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**Studies of the interaction between diabetes
family history, exercise, adiposity and
metabolic health**

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University
of Glasgow

ABSTRACT

The rising tide of obesity and type 2 diabetes has been recognised to have reached epidemic proportions. There is a significant burden of mortality and morbidity associated with the development of these conditions and current estimates suggest that the burden of disease in the next two decades is likely to place considerable strain upon healthcare systems, particularly in the developing world. The development of insulin resistance is a key contributor to the pathogenesis of type 2 diabetes, however the origin of insulin resistance is complex and it is currently unclear precisely how the inter-related components of this metabolic dysfunction are triggered. Obesity is also implicated in the pathogenesis of insulin resistance and represents a risk factor which is potentially modifiable by lifestyle interventions such as exercise and weight loss. Observational and prospective studies have also shown the benefits of lifestyle intervention in reducing the incidence of diabetes in those judged to be at greater risk.

People with a parental history of type 2 diabetes have an increased lifetime risk of diabetes and frequently display metabolic abnormalities which, despite persisting normoglycaemia, are evidence of a 'pre-diabetic' state and which may themselves carry increased morbidity and mortality. Observational studies suggest a greater difference in insulin sensitivity between active and sedentary offspring, compared to the difference between active and sedentary individuals with no diabetes family history. This is thought to represent an interaction between positive energy balance, a sedentary lifestyle and a 'thrifty genotype'. These observations suggest that individuals with a parental history of diabetes are more susceptible to the deleterious health effects of a sedentary lifestyle, but that they may be more responsive to an increase in physical activity. Exercise interventions can be expected to have positive effects upon metabolic health and adiposity however, the individual response to exercise is extremely variable. Other factors such as lifestyle alteration have been implicated in the difference between the observed and predicted response to exercise. Therefore, the aim

of this thesis was to examine the physical, metabolic and lifestyle differences between sedentary pre-menopausal women with a parent with type 2 diabetes and matched control subjects with no family history of the condition. In particular, this thesis aimed to explore the effect of an aerobic exercise intervention on metabolic health and body composition, whether the response to exercise is dependent upon a familial history of diabetes and the processes by which exercise might induce any observed changes.

In order to explore the impact of a sedentary lifestyle on women with, and without a family history of type 2 diabetes, thirty four pre-menopausal, sedentary women with a parental history of diabetes (Offspring) and thirty six matched women without a familial history of diabetes (Controls) were recruited. Assessments of body composition, insulin sensitivity, adipose tissue-derived hormone concentration, substrate utilisation, endothelial function by carotid-radial pulse wave velocity, cardiorespiratory fitness, diet and habitual physical activity were performed. Twenty eight Offspring subjects and thirty four matched Controls participated in a seven-week aerobic exercise intervention, training at 65-80 % of predicted maximal heart rate with incremental increases in training duration on a weekly basis. The previously described assessments were performed before and 15-24 hours after the intervention and in a subgroup of 19 Controls and 17 Offspring subjects, further assessments of insulin sensitivity, adipose tissue-derived hormone concentration, substrate utilisation and endothelial function were performed after a further three day period without exercise. In order to determine potential mediators of exercise-induced fat loss fifty five women participated in measurements of substrate utilisation, body composition, endothelial function, insulin sensitivity, cardiorespiratory fitness, dietary intake and habitual physical activity prior to, and after the seven week exercise intervention.

The findings from these studies confirmed that sedentary women with a family history of type 2 diabetes displayed lower insulin sensitivity than those without a parental history of

diabetes. In addition, insulin resistance in this group appears to be related to a greater sensitivity to the influence of adipose tissue, particularly circulating non-esterified fatty acids and adipose tissue-derived inflammatory cytokines. In Offspring alone, baseline insulin sensitivity was associated with plasma adiponectin concentration and negatively associated with circulating non-esterified fatty acid concentration. These associations may represent physiological attempts to compensate for developing insulin resistance. Offspring also displayed an augmented metabolic response to the exercise intervention in comparison to Controls. This study showed a 23% increase in post-intervention insulin sensitivity in Offspring with no significant increase in insulin sensitivity in Controls despite a similar improvement in cardiorespiratory fitness and adherence to the exercise regime. Improved post-intervention insulin sensitivity was accompanied by reduced circulating leptin, increased fat and decreased carbohydrate oxidation in both fasting and post-glucose states. No change in diet was observed but Offspring appeared to increase their level of habitual physical activity. The magnitude of change in insulin sensitivity was associated with a parental history of diabetes, but stronger associations were observed between baseline insulin resistance and an ability to reduce circulating leptin in response to exercise. Wide individual variation in fat mass change was observed in the response to exercise, and as expected the strongest predictor of exercise-mediated fat mass reduction was the net energy cost of the intervention. However, a change in fasting respiratory exchange ratio (RER), suggesting an increase in fat oxidation was also independently associated with reduced fat mass.

The combined findings of this thesis suggest that sedentary pre-menopausal daughters of people with type 2 diabetes are more insulin resistant and that this state is, in part, a consequence of heightened sensitivity to fatty acid and inflammatory cytokine release from adipose tissue. However, it would also appear that they represent a high-risk group who are susceptible to the insulin-sensitising effects of exercise and that this may be mediated by a metabolic pathway which involves reductions in circulating leptin concentrations. Finally, the ability to lose fat mass in response to exercise is related to the energy deficit incurred by the

activity but also by an individual's ability to shift fasting substrate utilisation towards fat oxidation. Public health strategies have traditionally focused upon lifestyle interventions which are directed at the population in general. However, awareness of the risks conferred by obesity and familial history of type 2 diabetes and the potential benefits of intervention may suggest that targeting public health resources towards these high-risk groups is a more appropriate and effective strategy.

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All people dream, but not equally. Those who dream by night in the dusty recesses of their minds wake in the day to find it was vanity. But the dreamers of the day are dangerous people, for they may act on their dreams with open eyes to make it possible.

T.E Lawrence.

In the beginning of the malady it is easy to cure but difficult to detect, but in the course of time, not having been either detected or treated in the beginning, it becomes easy to detect but difficult to cure.

Niccolò Macchiavelli (1513).

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DECLARATION OF PUBLICATIONS

Unless otherwise indicated by acknowledgement or reference to published literature the work contained herein is that of the author.

The findings of some of the studies described in this thesis have been published as follows:

Published research papers

Barwell ND, Malkova D, Moran CN, Cleland SJ, Packard CJ, Zammit VA, Gill JM. (2008). Exercise training has greater effects on insulin sensitivity in daughters of patients with type 2 diabetes than in women with no family history of diabetes. *Diabetologia* 51(10):1912-9.

Barwell ND, Malkova D, Leggate M, Gill JM. (2009)

Individual responsiveness to exercise-induced fat loss is associated with change in resting substrate utilization. *Metabolism*. 58(9):1320-8.

LIST OF ABBREVIATIONS

T2D	Type 2 diabetes mellitus
$\dot{V}O_{2\max}$	Maximal oxygen uptake
3-OHB	3-hydroxybutyrate
NEFA	Non-esterified fatty acid
PWV	Pulse wave velocity
BP	Blood pressure
TG	Triglyceride
HDL	High density lipoprotein
LDL	Low density lipoprotein
VLDL	Very-low density lipoprotein
sdLDL	Small, dense low density lipoprotein
BMI	Body mass index
RMR	Resting metabolic rate
PGMR	Post-glucose metabolic rate
RER	Respiratory exchange ratio
CHO	Carbohydrate
SFA	Saturated fatty acids
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids

RPE	Rate of perceived exertion
IFG	Impaired fasting glycaemia
IGT	Impaired glucose tolerance
PPAR	Peroxisome proliferator-activated receptor
GNG	Gluconeogenesis
GL	Glycogenolysis
HGP	Hepatic glucose production
AMPK	Adenosine monophosphate-kinase
LPL	Lipoprotein lipase

CHAPTER 1

INTRODUCTION & LITERATURE REVIEW

1.1 Introduction

This chapter aims to provide the relevant scientific background to the studies in this thesis, additionally it aims to establish the theoretical basis for these studies. This chapter begins with an overview of type 2 diabetes mellitus, insulin resistance, obesity and the public health impact of these conditions. Following this is a more detailed consideration of the role of insulin resistance and obesity as co-founders in the development of premature cardiovascular disease. Finally the aetiology of insulin resistance is explored, including mechanisms by which insulin resistance can be modified and which may lead to the reduction in risk of both the development of type 2 diabetes and cardiovascular disease.

1.2 Type 2 diabetes mellitus and other states of impaired glucose homeostasis

Diabetes mellitus is a disease of metabolic dysfunction, characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (WHO Consultation 1999) (Table 1.1). Type 2 diabetes (T2D) is a disease of insulin resistance, although defects in pancreatic beta-cell function, mass and insulin secretion are generally also required to achieve the degree of hyperglycaemia which is diagnostic of the condition (Ferrannini 1998; Kahn 2003; McGarry 2002; Petersen & Shulman 2006; Reaven 1988). Chronic hyperglycaemia is associated with the development of microvascular disease which, through an effect on the microvascular beds of the retina, renal parenchyma, and both peripheral and autonomic nervous systems may give rise to symptomatic or occult complications (The DCCT Research Group 1993; UKPDS Study Group 1998). However, the process of microvascular disease is not confined to these vascular beds (Rizzoni et al. 2001). Indeed, microvascular disease is recognised to be present early in the pathogenesis of T2D (Jaap, Shore, & Tooke 1997), at the level of the skeletal

muscle microcirculation and is closely associated with the degree of insulin resistance (Sydow, Mondon, & Cooke 2005). Of additional clinical relevance, macrovascular diseases such as coronary artery disease, cerebrovascular disease and occlusive peripheral vascular disease are more common in patients with diabetes (Stamler et al. 1993).

Other states of impaired glucose homeostasis have been categorised and although these states do not share the chronic hyperglycaemia of T2D, many of the fundamental metabolic abnormalities found in T2D are common to these ‘pre-diabetic’ conditions. Impaired Glucose Tolerance (IGT) and Impaired Fasting Glycaemia (IFG) are both associated with an increased risk of developing T2D (Unwin et al. 2002). However although these conditions can exist separately, they may also overlap (Table 1.1) and represent stages on the continuum of insulin resistance and dysglycaemia (Bi et al. 2010; Ning et al. 2010). The risk of developing T2D from either isolated IFG, isolated IGT or combined IFG-IGT is dependent on the background risk within a given population: however, the development of altered glucose homeostasis is associated with a significant increase in the risk of progression to T2D and the combination of IFG and IGT may be associated with an increased relative risk of the order of 4-15 fold over a period of 5 – 10 years (Unwin et al. 2002).

Table 1.1 Diagnostic criteria for diabetes mellitus, IFG and IGT (WHO Consultation 1999).

	Fasting plasma glucose (mmol.l⁻¹)	2 – hour post-glucose load plasma glucose (mmol.l⁻¹)
Diabetes mellitus	≥ 7.0	≥ 11.1
Isolated IFG	6.1 – 6.9	-
Isolated IGT	≤ 6.1	7.8 – 11.0
Combined IFG & IGT	6.1 – 6.9	7.8 – 11.0

The fasting plasma glucose threshold of 7.0 mmol.l^{-1} is a diagnostic indicator of diabetes, but is also the threshold at which microvascular complications begin to occur (WHO Consultation 1999). There is no evidence for increased risk of microvascular complications in patients with IFG or IGT (Unwin et al. 2002), although subclinical microvascular dysfunction is present in these dysglycaemic states and is evident early in the pathogenesis of insulin resistance (Jessani, Millane, & Lip 2009). However, macrovascular complication risk does not appear to be limited by a glycaemic threshold. There is evidence from observational studies that glycaemia is independently associated with cardiovascular events and that post-prandial glycaemia is more strongly associated with cardiovascular events than fasting glycaemia (Hanefeld et al. 2000;The DECODE Study Group 1999;The DECODE Study Group 2001).

The term 'Metabolic Syndrome' (Ferrannini et al. 1991;Haller 1977;Reaven 1992) has been used to define a group of risk factors which are common to both the development of T2D and cardiovascular disease (Laws & Reaven 1993;Lebovitz 2006). The exact definition of the components of the Metabolic Syndrome has been debated, leading to a number of different definitions and a number of adjustments for ethnicity (Table 1.2). Regardless of the definition, emphasis is placed on the combination of central obesity, dyslipidaemia, hypertension and dysglycaemia (Alexander et al. 2003;International Diabetes Federation 2005;WHO Consultation 1999). Identification of the Metabolic Syndrome is of clinical benefit in raising awareness of both cardiovascular risk and risk of subsequent development of T2D (Grundy et al. 2006;Saely et al. 2006;Sattar et al. 2003).

Table 1.2 Defining criteria for Metabolic Syndrome.

	WHO	NCEP ATP III	IDF
Insulin resistance	<div> ↑ Insulin levels <u>or</u> ↑ Fasting plasma glucose <u>or</u> ↑ Post-prandial glucose </div>	Fasting blood glucose $\geq 6.1 \text{ mmol.l}^{-1}$	Fasting plasma glucose $\geq 5.6 \text{ mmol.l}^{-1}$ <u>or</u> Known T2D
Dyslipidaemia	<div> $\text{TG} > 1.7 \text{ mmol.l}^{-1}$ <u>or</u> $\text{HDL} < 1.94 \text{ mmol.l}^{-1}$ </div>	<div> $\text{TG} > 1.7 \text{ mmol.l}^{-1}$ $\text{HDL} < 1.03 \text{ mmol.l}^{-1} \text{ ♂}$ $< 1.29 \text{ mmol.l}^{-1} \text{ ♀}$ </div>	<div> $\text{TG} > 1.7 \text{ mmol.l}^{-1*}$ $\text{HDL} < 1.03 \text{ mmol.l}^{-1} \text{ ♂}^*$ $< 1.29 \text{ mmol.l}^{-1} \text{ ♀}^*$ </div>
Blood pressure	$\geq 140/90^*$	$> 130/85$	<div> $\text{SBP} \geq 130^*$ $\text{DBP} \geq 85^*$ </div>
Obesity	<div> Waist-Hip ratio > 0.9 BMI 30 Waist circ $> 94\text{cm}$ </div>	<div> Waist circ $> 102 \text{ cm } \text{♂}$ $> 88 \text{ cm } \text{♀}$ </div>	<div> $\text{Waist circ} \geq 94 \text{ cm } \text{♂}$ $\geq 80 \text{ cm } \text{♀}$ </div>

Shaded boxes indicate that condition is required for diagnosis. For WHO & IDF classifications, additional 2 criteria must be met. For NCEP classification a combination of any 3 factors is diagnostic. * or treatment for stated condition (Alexander et al. 2003;International Diabetes Federation 2005;WHO Consultation 1999).

Metabolic syndrome, IFG and IGT criteria allow identification of individuals ‘at-risk’ of developing premature cardiovascular disease, type 2 diabetes or both. Although not characterised as disease states in themselves, they serve to focus clinical perspective on aggressive risk factor management. Early lifestyle intervention with dietary and physical activity advice is recommended (Grundy et al. 2006;Knowler et al. 2002;Orchard et al.2005;Pan et al. 1997;Tuomilehto et al. 2001), with additional use of pharmacological agents to treat hypertension and dyslipidaemia. To date, use of pharmacological agents to treat dysglycaemia has been extensively investigated, with evidence to suggest that progression from IGT and IFG to T2D can be delayed and possibly prevented (Scheen 2007). There is however, a lack of long-term data on the efficacy of these interventions (Grundy et

al. 2006) and doubt about the cost-effectiveness of alternatives (Eddy, Schlessinger, & Kahn 2005;Herman et al. 2005;Johansson et al. 2009).

1.3 The rising tide of obesity and type 2 diabetes

The global prevalence of obesity is increasing (Ford & Mokdad 2008), and from a long-term public health perspective, this situation is likely to deteriorate further given the burden of obesity in children and adolescents (Krassas et al. 2001). The obesity epidemic has been paralleled by a global increase in the prevalence of T2D (King, Aubert, & Herman 1998;Wild et al. 2004). Increases in diabetes may be partially related to the aging population in developed countries (Wild et al. 2004), however the largest increase in diabetes is projected to occur in the 45 – 64 year age group in developing countries which is related to increased urbanised lifestyle and associated obesity in this age group (King et al. 1998;Wild et al. 2004). Recent estimates of a global prevalence of 366 million patients with diabetes by 2030 may be conservative based on assumptions that the current prevalence of obesity remains constant (Wild et al. 2004). Obesity is a well recognised risk factor for the development of T2D (Barrett-Connor 1989;Edelstein et al. 1997;Ohlson et al. 1985;Ohlson et al. 1988) and may account for 80-90% of all T2D incidence (Astrup & Finer 2000), however not all those persons who become obese will develop T2D and both insulin resistant pre-diabetic states and T2D may occur in non-obese individuals (St Onge, Janssen, & Heymsfield 2004).

1.4 Populations at risk of type 2 diabetes

The population risk of diabetes is not constant between ethnic groups. Ethnically diverse countries such as the USA allow the analysis of the impact of ethnicity on the risk of developing diabetes and comparison of the diabetogenic effects of a ‘Westernised’ lifestyle (sedentary physical activity levels, high energy diet rich in fat and refined carbohydrate) on ethnic groups by comparison with their parent populations (Nakanishi et al. 2004;Schulz et

al. 2006). McBean and colleagues (2004) observed a higher diabetes prevalence in Black, Hispanic and Asian Americans compared with Caucasian Americans, and recorded the greatest increases in rates of diabetes amongst the Asian Americans over a seven year period (McBean et al. 2004). Although some of the increased risk in ethnic groups may be related to greater obesity levels (Centers for Disease Control and Prevention (CDC) 2009; Lorenzo et al. 2009), compared to Caucasians, other ethnic populations continue to display increased risk in normal weight individuals (Zhang, Wang, & Huang 2009). Maskarinec and co-workers (2009) describe an approximate two-fold increase in diabetes prevalence in all ethnic groups compared to Caucasians, even after adjustment for body mass index (BMI) (Maskarinec et al. 2009).

As previously stated, obesity is not the sole determinant of T2D. A multiple regression analysis of over 100,000 person-years of observational data in the Physicians' Health Study suggests that a sedentary lifestyle may account for 25% of all diabetes incidence (Manson et al. 1992). In addition, comparative ethnic studies have identified increased risk of diabetes in populations exposed to a Westernised lifestyle, compared with genetically similar populations who have higher levels of habitual physical activity and lower energy diets (Nakanishi et al. 2004; Schulz et al. 2006). Since these populations are genetically identical, the increased risk of T2D must be the result of an interaction between a genetic susceptibility to T2D and an environment which promotes diabetes (Figure 1.1).

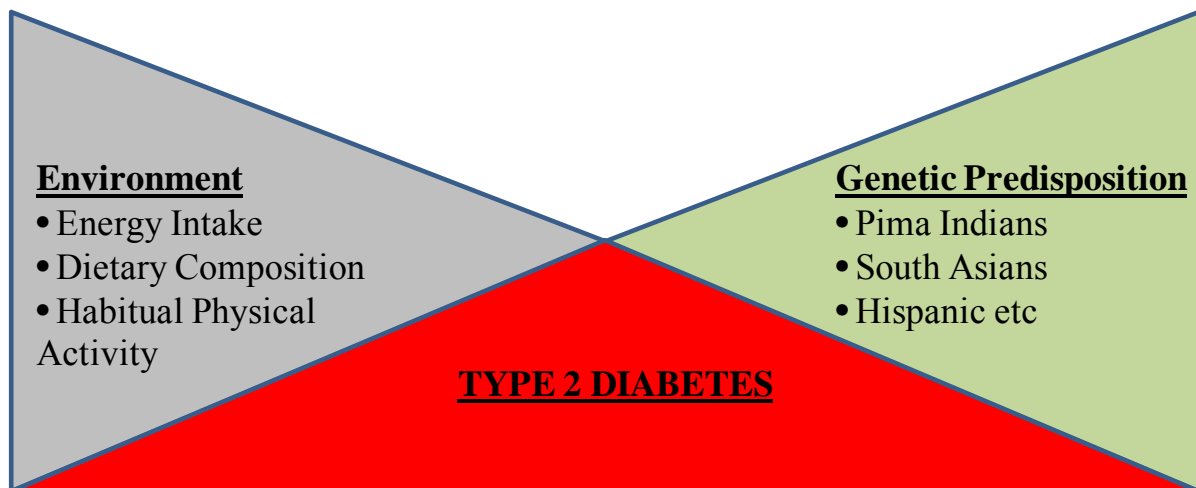


Figure 1.1 Gene-environment interaction in the development of T2D.

The paradigm of the gene-environment interaction is observed in the Pima Indian communities in the USA and in Mexico. Mexican Pima Indians lead a physically demanding non-mechanised, agricultural lifestyle, and have no greater prevalence of obesity or diabetes than non-Pima Mexicans living in the same area (Valencia et al. 1999). Five-fold increases in the prevalence of both obesity and T2D have been described in American Pima Indians, despite higher energy intake in the Mexican Pimas (Schulz et al. 2006; Valencia et al. 1999). A significant difference was observed between physical activity levels in the Mexican and American populations, with American Pima Indians participating in less than 5 hours of activity per week (a 4-fold difference) (Schulz et al. 2006; Valencia et al. 1999). More detailed assessment of the dietary habits of the American Pima Indians revealed a similar energy intake and macronutrient composition to the general American population but compared to the Mexican Pimas, a greater percentage of the calories consumed were derived from fat (Smith et al. 1996).

Further evidence of a gene-environment interaction can be seen in other groups. The Australian Aboriginals of the Torres Strait Islander community display rates of obesity and

diabetes which are 3-fold and 6-fold higher than the general Australian population (Leonard et al. 2002). Canadian Oji-Cree Indians, like American Pimas, display an extremely high prevalence of T2D and a close relationship between development of the condition and a Westernised lifestyle (Hegele et al. 2003). The common factor in all these geographically disparate communities is a relatively rapid change in lifestyle; from a traditionally high level of habitual physical activity and a diet composed of high fibre and low fat, to a sedentary lifestyle with equivalent total energy intake but a dietary composition high in refined carbohydrate and fat. Specific 'pro-diabetes' gene mutations have been observed in Oji-Cree (Hegele et al. 2003) and Pima Indians (Ma et al. 2008). The rapid increase in diabetes prevalence in these communities cannot be attributed to genetic factors which are likely to have been present within these groups for generations. However, given the 'sudden' rise in diabetes, it is likely that a longstanding genetic predisposition towards T2D (which presumably previously conferred a selection advantage) has combined with a 'diabetogenic' lifestyle. This so-called 'thrifty genotype' has previously been described (Neel 1962), and considered in the context of diabetes (Booth, Chakravarthy, & Spangenburg 2002).

Other groups who are at risk of T2D have been identified. First-degree relatives of patients with T2D have approximately three times the risk of developing diabetes than matched controls with no family history of the disease (Ohlson et al. 1988;Kobberling & Tillil. 1982). In addition, they appear to display metabolic manifestations of this risk, in the form of insulin resistance, long before the development of dysglycaemia (Ezenwaka, Davis, & Offiah 2001;Humphriss et al. 1997;Perseghin et al. 1997;Warram et al. 1990). This risk appears to be particularly evident in sedentary people with a first-degree relative with T2D (Higgins et al. 2005). Sedentary offspring of people with diabetes display 'pre-diabetic' metabolic abnormalities such as reduced insulin sensitivity (Ahn et al. 2004;Ekelund et al. 2009;Ekelund, Griffin, & Wareham 2007) and are more likely to manifest higher waist circumferences and dyslipidaemia than active offspring (Higgins et al. 2005). This increased metabolic risk appears to be partially attenuated in offspring who are active (Ahn et al.

2004;Ekelund et al. 2009) it is however unclear whether intervention in sedentary individuals with established pre-diabetic metabolic changes would lead to a significant alteration in metabolic profile and the subsequent risk of developing T2D. One of the aims of this thesis is to establish the extent to which an exercise intervention influences the metabolic profile which is associated with the combination of a sedentary lifestyle and a parental history of T2D.

1.5 Cardiovascular risk and insulin-resistant states

The diagnosis of T2D is understood to confer up to a four-fold increase in cardiovascular risk (Kannel & McGee 1979;Stamler et al. 1993). Indeed, it has been suggested that the diagnosis of T2D should be viewed as a cardiovascular risk equivalent to that of a patient with a previous myocardial infarction (Haffner et al. 1998;Whiteley et al. 2005). However, recent meta-analysis would not support this conclusion and in fact suggests that individual patient risk factors should be assessed, rather than a ‘blanket’ assumption that the onset of T2D automatically confers increased cardiovascular risk (Bulugahapitiya et al. 2009). The method of this meta-analysis is not able to assess the specific cardiovascular impact of T2D on women and ethnic groups (Bulugahapitiya et al. 2009) and there is some evidence that T2D may have a relatively greater cardiovascular impact upon women compared to men (Becker et al. 2003;Hu et al. 2001;Mak & Haffner 2003), and South Asians compared to Caucasians (Patel et al. 2008). For South Asians it is unclear whether the increased cardiovascular risk is related to a specific effect of diabetes in this population, or simply related to the increased prevalence of T2D (Tziomalos et al. 2008).

Whilst T2D may have a heterogenous effect on cardiovascular risk which is difficult to quantify, it is clear that diabetes does contribute to cardiovascular mortality and morbidity (Stamler et al. 1993) and that cardiovascular risk is likely to be related to the duration of the

condition (Shah et al. 2009a). In addition to increasing risk of cardiovascular events, T2D is also associated with higher first-event mortality (Miettinen et al. 1998) and high rates of recurrent events (Giorda et al. 2008). Miettinen and colleagues (1998) also observed that the increased risk conferred by co-existing diabetes and vascular events was greater in women than in men (Miettinen et al. 1998). Additionally, patients with diabetes are estimated to suffer from cardiovascular events at a much younger age, possibly 15 years before equivalent non-diabetic patients (Booth et al. 2006). Patients with diabetes also suffer from greater morbidity after cardiovascular events (Murcia et al. 2004;Oliveira et al. 2002;Stone et al. 1989;Yu et al. 2000).

Increased cardiovascular risk is also observed in other insulin resistant states. The metabolic syndrome is associated with increased prevalence of coronary heart disease (Alexander et al. 2003;Lakka et al. 2002) and increased incidence of all cardiovascular events (Lakka et al. 2002;McNeill et al. 2005;Rutter et al. 2005). Malik and colleagues (2004) showed that cardiovascular disease and total mortality was increased in patients with the metabolic syndrome, and that risk was increased even when only one or two of the diagnostic criteria were met (Malik et al. 2004).

Rutter and co-workers suggested that the increased cardiovascular risk observed in metabolic syndrome patients was independently associated with low insulin sensitivity, after correction for other diagnostic criteria (Rutter et al. 2005). This may suggest that the key metabolic abnormality which drives increased risk in these patients is insulin resistance (Saely et al. 2005). Liese and colleagues previously highlighted the role of insulin resistance (categorised as hyperinsulinaemia) as a major determinant in the subsequent development of metabolic syndrome (Liese et al. 1997). Confirmatory factor analysis by Shen and colleagues (2003) suggested that insulin resistance and obesity were the primary contributors to the development of metabolic syndrome, and by extension the underlying risk of subsequent

diabetes and vascular disease (Shen et al. 2003). More recent confirmatory factor analysis suggests that one key metabolic driver could be the foundation for the multiple abnormalities (Pladevall et al. 2006).

Further evidence that insulin resistance is an important cardiovascular risk factor is evident. The DECODE Study Group highlighted the association between IGT and mortality (The DECODE Study Group 1999;The DECODE Study Group 2001). Additionally, Blake and colleagues (2004) identified increased coronary heart disease (CHD) in individuals with IGT (Blake et al. 2004), whilst Brunner and co-workers (2006) observed a linear increase in CHD at post-challenge glucose concentrations of greater than 4.6 mmol.l⁻¹ (Brunner et al. 2006). Hu and colleagues (2002) also described greater risk of cardiovascular events in ‘pre-diabetic’ women who subsequently developed T2D (Hu et al. 2002). Even within patients with established T2D, insulin resistance is independently associated with cardiovascular risk (Bonora et al. 2002).

1.6 Common metabolic cofounders of cardiovascular risk in insulin resistant states

Although insulin resistance is a key cofounder of metabolic syndrome, T2D and the attendant cardiovascular risk, other abnormalities such as dyslipidaemia (Taskinen 2005), obesity (Grundy 2002;Han et al. 2002) and chronic low-grade inflammation (Pradhan et al. 2001;Sattar et al. 2003) are frequently present in affected individuals. These factors may interact with, or be dependent upon, insulin resistance producing a complex metabolic state which predisposes to both atherosclerosis and T2D (Figure 1.2).

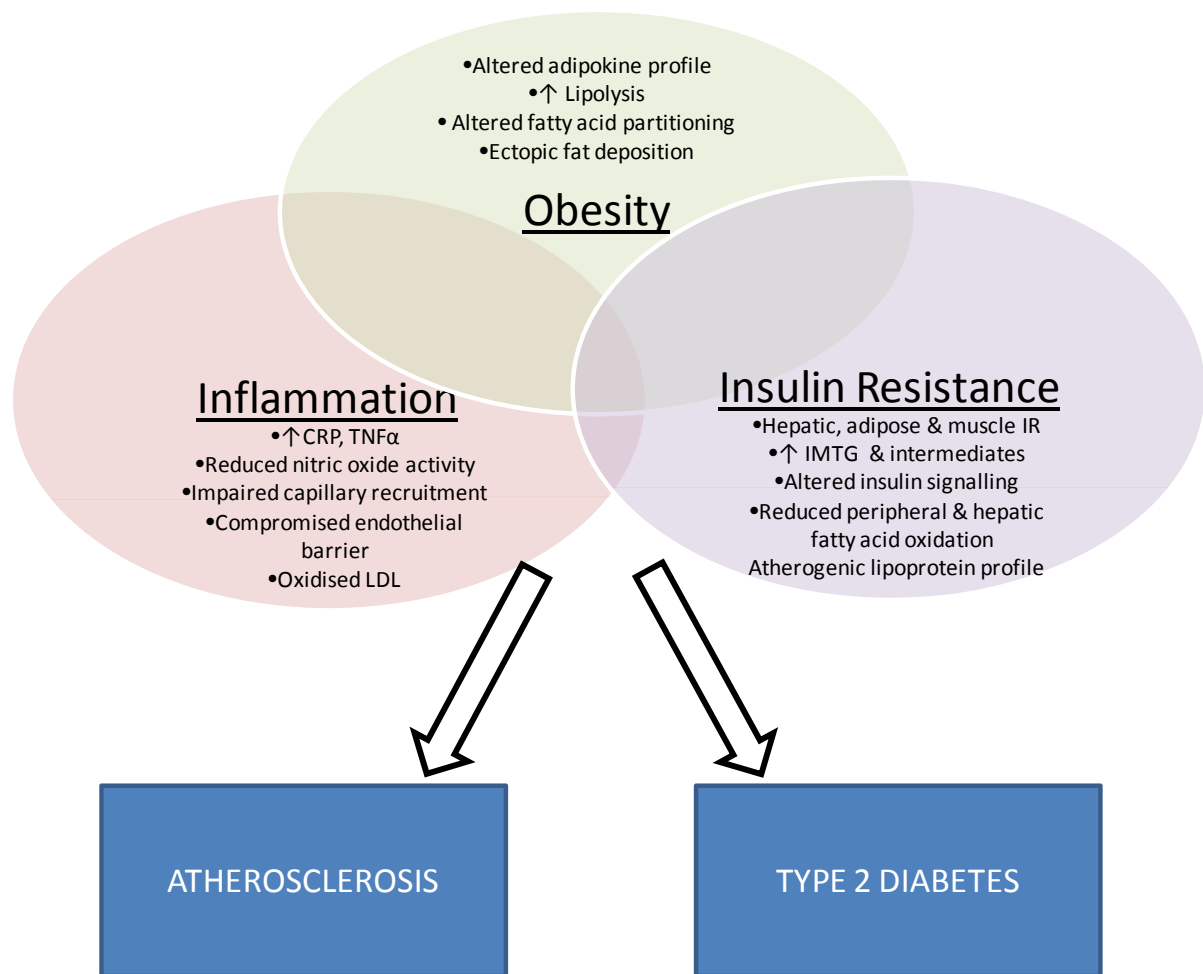


Figure 1.2 Obesity, insulin resistance and chronic inflammation have complex interactions in the development of atherosclerosis and T2D.

Appreciation of the importance of cardiovascular risk in patients with, or at risk of, T2D is recognised to be of paramount importance. A shift in diabetes care has occurred in the last two decades, from a glucocentric model with concentration on detection of early microvascular disease to a model which includes these traditional goals but also targets aggressive treatment of macrovascular risk. Clinical targets for the optimal management of T2D include early detection of cardiovascular risk markers and aggressive treatment (American Diabetes Association 2008). Further exploration of the role of diabetes in the development of cardiovascular disease is warranted, with specific focus on risks in women

and non-Caucasians. Pre-menopausal women have a low risk of cardiovascular events and are therefore relatively understudied with respect to metabolic risk factors which might contribute to heightened cardiovascular risk. In view of the increased relative risk of cardiovascular disease in women who develop diabetes, this thesis aims to examine pre-menopausal daughters of patients with T2D in an attempt to further characterise the metabolic processes which might contribute to later diabetes and cardiovascular disease.

1.7 Type 2 diabetes: Insulin resistance or beta-cell dysfunction?

Both insulin resistance and reduced insulin secretion are recognised in T2D (Kahn 2003). The pancreatic beta-cell has been traditionally considered to display adaptive secretory responses to insulin resistance, by augmenting insulin secretion to maintain normoglycaemia (Cerasi 1995; Taylor, Accili, & Imai 1994). Beta-cell failure has therefore been thought to be a relatively late occurrence in T2D, signifying a failure of both this adaptive response and the ability to maintain normal blood glucose. It is now accepted that beta-cell dysfunction occurs earlier in the natural history of the development of T2D, although this toxicity is still a consequence of the complex metabolic state found in insulin resistance with direct effects mediated by circulating non-esterified fatty acids (NEFA) (Kahn 2003). It is likely therefore, that insulin resistance remains the key metabolic abnormality in the development of T2D and that this, in concert with the metabolic effects of obesity, drives early beta-cell toxicity (Groop 1999).

1.8 The aetiology of insulin resistance

Given the importance of insulin resistance in the development of type 2 diabetes and its independent associations with cardiovascular disease, early intervention to prevent or reverse its progression may have significant public health benefits by the reduction of these chronic diseases (Lopez & Murray 1998). The state of insulin resistance is characterised by a reduced

ability of insulin to suppress hepatic glucose production and to promote peripheral glucose disposal. Type 2 diabetes occurs when this condition is accompanied by a failure of the pancreatic beta-cell to secrete enough insulin to overcome the degree of insulin resistance (McGarry 2002). The insulin resistant state is associated with metabolic dysfunction in adipose tissue, hepatic, skeletal muscle tissue and the vascular endothelium. These changes are both a consequence of, and contribute to, insulin resistance and it is difficult to separate metabolic cause, from effect.

1.8.1 Insulin resistance and adipose tissue

Adipose tissue is a metabolically active site of energy balance and hormone synthesis (Havel 2004). The adipocytokines produced by adipose tissue have paracrine and endocrine function, therefore adipose tissue has the ability to influence other tissues such as liver and skeletal muscle through both the dynamic flux of fatty acids and tissue-derived hormones. An enlarged adipose tissue compartment is associated with increased lipolysis and increased circulating non-esterified fatty acids (NEFA) (Boden 1997; Delarue & Magnan 2007) and is indicative of adipose tissue insulin resistance (Delarue & Magnan 2007). Increased NEFA induces chronic skeletal muscle insulin resistance through both reduced carbohydrate oxidation and reduced insulin stimulated glucose uptake (Boden 1997; Boden & Shulman 2002). In an insulin resistant state, adipose tissue fails to suppress lipolysis in response to circulating insulin and may lead to inappropriate release of NEFA in the context of a post-prandial state, producing further 'lipid overflow' to the liver and skeletal muscle (Arner 2005). In addition, NEFA induce hepatic insulin resistance characterised by unchecked hepatic glucose production (Roden et al. 2000), although there may be a greater contribution to hepatic glucose production by NEFA-induced elevation of circulating insulin and glucagon (Chen, Iqbal, & Boden 1999). However, since NEFA concentrations are not always elevated in insulin resistant states, the lipotoxicity may be related to relatively reduced fat oxidative capacity in target organs (Corpeleijn et al. 2009). Alternatively, post-prandial NEFA

concentrations may have relatively greater impact upon insulin sensitivity and therefore elevated circulating NEFA in response to dietary fats could be implicated in the 'lipid overflow' (Jackson et al. 2005).

Further contribution to 'lipid overflow' may occur in the post-prandial state in insulin resistant subjects. Lipoprotein lipase (LPL) is responsible for hydrolysis of circulating triglyceride, releasing fatty acids which can be taken up by adipose tissue or skeletal muscle. In health, LPL expression on the vascular endothelium of adipose tissue is enhanced by insulin, promoting uptake of fatty acids in the fed state. Skeletal muscle LPL is not influenced by insulin and uptake of fatty acids is dependent on circulating plasma concentrations (Frayn, Arner, & Yki-Jarvinen 2006). Type 2 diabetes, insulin resistance and carbohydrate-rich diets produce greater post-prandial elevations in triglycerides than in insulin sensitive and carbohydrate restricted subjects (Karpe 1997; Kim et al. 2001a). Higher post-prandial NEFA concentrations are attributable to unsuppressed lipolysis in adipose tissue and the insulin-resistant liver also fails to suppress VLDL-triglyceride secretion (Frayn, Arner, & Yki-Jarvinen 2006). Therefore insulin-resistant adipose tissue and liver combine to provide skeletal muscle with both NEFA and fatty acid-rich lipoproteins which increase intramyocellular triglyceride through the unrestricted actions of skeletal muscle LPL.

Adipo(cyto)kines have a complex relationship with insulin sensitivity and appear to influence a number of metabolic systems which can contribute to the modulation of insulin sensitivity (Havel 2004). Obesity has been described as a chronic low-grade inflammatory condition (Hotamisligil et al. 1995). Adipokines mediate stimulation of both inflammation and insulin resistance in a paracrine fashion in adipose tissue and by an endocrine effect on skeletal muscle, liver and endothelium (Gustafson 2010). Chronic overfeeding results in expansion of adipose tissue pools due to enlargement of adipocytes (Spalding et al. 2008). Enlarged adipocytes alter their adipokine secretory profile, increasing expression of interleukin-6 (IL-

6) which stimulates lipolysis and macrophage infiltration of adipose tissue (Heilbronn & Campbell 2008). Spalding and colleagues have shown increased adipocyte turnover in obese patients (Spalding et al. 2008) and it has been suggested that enlarged adipocytes which become hypoxic and subsequently necrotic also stimulate chronic inflammation within adipose tissue (Heilbronn & Campbell 2008). The combination of enlarged adipocytes and increased adipose tissue macrophages results in a positive feedback cycle of chronic inflammation characterised by increased IL-6, Tumour Necrosis Factor- α (TNF α), plasminogen activator inhibitor-1 (PAI-1) and other cytokines which have local effects on adipose tissue and systemic effects (Vettor et al. 2005; Berg & Scherer 2005). Adipocytes release cell adhesion molecules such as inter-cellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) which recruit circulating immune cells into the vascular wall (Andersson et al. 2008). Adipose tissue bound macrophages and adipocytes are responsible for TNF α production which mediates impaired insulin signalling through activation of the nuclear factor kappa-B (NF- κ B) transcription factor (Wagenmakers et al. 2006). Activation of NF- κ B by the same stimulus has further effects on the endothelium leading to increased expression of cell adhesion molecules and by a separate pathway, TNF α -mediated superoxide formation leads to reduced nitric oxide bioavailability and further endothelial damage (Wagenmakers et al. 2006).

Leptin is correlated with adipose tissue mass, has local pro-inflammatory effects and increased circulating leptin is associated with insulin resistance (Nyholm et al. 1997; Wannamethee et al. 2007). Additionally, obesity is associated with low circulating adiponectin and is correlated with insulin resistance (Hotta et al. 2000; Weyer et al. 2001; Yamauchi et al. 2001). Both leptin and adiponectin influence insulin sensitivity directly but also contribute to endothelial function and therefore influence insulin sensitivity indirectly through modulation of the chronic inflammatory state which co-exists with insulin resistance (Berg & Scherer 2005).

1.8.2 Insulin resistance in skeletal muscle

Skeletal muscle is the most important site of insulin-stimulated glucose disposal (DeFronzo et al. 1981) and is the main site of peripheral insulin resistance (DeFronzo et al. 1985). Skeletal muscle insulin resistance is associated with increased intramyocellular lipid accumulation (Krssak et al. 1999) and impaired insulin stimulated glucose uptake and oxidation (Kelley & Mandarino 2000; Turcotte & Fisher 2008). It is suggested that chronically increased circulating NEFA concentrations lead to increased intramuscular triglyceride and lipid intermediate deposition which in turn interfere with both the intracellular insulin signalling cascade (Wolf 2008) and subsequent GLUT4 dependent insulin-stimulated glucose uptake (Delarue & Magnan 2007). The so-called ‘athlete’s paradox’ would appear to cast doubt over the role of intramyocellular lipid in insulin resistance, since trained subjects who are highly insulin sensitive have high levels of intramyocellular lipid (IMCL) (Goodpaster et al. 2001). However, it is suggested that it is the dynamic turnover of IMCL rather than the total volume which is the important factor in determining the impact of IMCL on insulin sensitivity (Corcoran, Lamon-Fava, & Fielding 2007).

The mechanism by which ‘static’ IMCL contributes to insulin resistance is incompletely understood but appears to be associated with reduced insulin signalling, GLUT4 expression, glucose uptake, lipid oxidation and glycogen synthesis (Corcoran, Lamon-Fava, & Fielding 2007; Zierath, Krook, & Wallberg-Henriksson 2000). Both increased circulating plasma NEFA and IMCL concentrations produced reduced insulin-stimulated glucose uptake and glycogen synthesis (Boden 1997; Roden et al. 1999; Savage et al. 2007). Specifically, lipid intermediate metabolites (ceramides, long-chain fatty acyl-CoA and diacylglycerol (DAG)) are implicated in the molecular processes which cause skeletal muscle insulin resistance. Diacylglycerol is understood to activate serine/threonine kinases (protein kinase C (PKC) family) which phosphorylate and subsequently reduce the activity of upstream components of the insulin signalling pathway such as the insulin receptor and insulin receptor substrate-1

(IRS-1) (Bonen, Dohm, & van Loon 2006; Petersen & Shulman 2006). A reduction in the activity of the insulin signalling cascade leads to lower insulin-stimulated glucose uptake, protein kinase C also inactivates glycogen synthase kinase 3 further reducing skeletal muscle glycogen storage (Delarue & Magnan 2007). Ceramides inhibit the activation of Akt/Protein Kinase B (Akt/PKB) at a point 'downstream' of phosphatidylinositol 3-kinase (PI3K) resulting in downstream disruption of the insulin signalling cascade (Stratford et al. 2004). Stratford and colleagues observed ceramide-mediated disruption of Akt/PKB activation by two mechanisms (firstly Akt/PKB membrane translocation, secondly Akt/PKB dephosphorylation) in 3T3-L1 preadipocytes and the same mechanism is likely in skeletal muscle (Strackowski & Kowalska 2008). Inhibition of ceramide synthesis and improved insulin sensitivity in animals is associated with increased Akt/PKB activity and in humans, exercise-mediated reductions in skeletal muscle ceramide have been associated with improved insulin sensitivity (Strackowski & Kowalska 2008). It has been hypothesised that IMCL-related increases in PKC activity may lead to activation of the transcription protein nuclear factor κ -B (NF κ B) which suppresses PI3K function, this has been observed in skeletal muscle in rodents but not, to date, in human skeletal muscle (Itani et al. 2002; Kim et al. 2001b).

Turcotte and Fisher (2008) suggest that individuals with insulin resistance may exhibit a constitutive increase in fatty acid binding proteins (such as CD36) leading to increased myocyte fatty acid incursion (Turcotte & Fisher 2008). In addition, fat oxidation is impaired in insulin resistant skeletal muscle, contrary to the state predicted by Randle's hypothesis. Insulin and glucose increase skeletal muscle content of malonyl CoA, which is synthesised in muscle from acetyl CoA by acetyl CoA carboxylase-2 (ACC2). The accumulation of malonyl CoA may reduce fatty acid oxidation by inhibition of carnitine palmitoyl transferase-1 (CPT1) and subsequent accumulation of long-chain fatty acids (such as palmitate) have been shown to induce skeletal muscle insulin resistance by inhibition of Akt/PKB (Bonen, Dohm, & van Loon 2006; Kelley & Mandarino 2000; Wolf 2008). In contrast with hepatic tissue,

skeletal muscle has low levels of fatty acid synthase (FAS) therefore despite increased concentrations of malonyl CoA, rates of fatty acid synthesis *de novo* are low in skeletal muscle (McGarry 2002). Accumulation of long-chain fatty acids or intermediates are therefore derived from circulating lipids or stored IMCL (Koves et al. 2008). Muscle biopsies from insulin resistant subjects treated with the peroxisome proliferator-activated receptor γ (PPAR γ) agonist rosiglitazone show restoration of malonyl CoA and ACC2 activity and increased fat oxidation (Bandyopadhyay et al. 2006). Insulin resistant subjects are recognised to have reduced density of skeletal muscle mitochondria and this has been considered to be important in the impairment of both fatty acid oxidation and ATP synthesis (Petersen, Dufour, & Shulman 2005). However, the idea of a primary mitochondrial deficiency being responsible for skeletal muscle insulin resistance appears to be inconsistent with the functional reserve of the mitochondria, the normal basal creatine phosphate/ATP ratio observed in insulin resistant subjects and apparently preserved oxidative function in severely insulin resistant diabetic subjects (Holloszy 2009; Nair et al. 2008). It has been suggested that reduced insulin-mediated ATP synthesis is in fact a consequence of local and central insulin resistance, without an underlying mitochondrial defect (Wagenmakers 2005).

1.8.3 Insulin resistance and endothelial dysfunction

Insulin is a vascular hormone and in healthy individuals, it promotes vasodilatation (Baron 1994; Cleland et al. 1999). Therefore, insulin-mediated glucose uptake in skeletal muscle is partly dependent on the vascular actions of insulin, which maintains blood flow through the vascular beds as well as the direct metabolic effect of insulin on GLUT4 translocation and glucose uptake (Laakso et al. 1990; Serne et al. 2006). There is debate about the validity of the relationship between vascular and metabolic actions of insulin since the vasodilating effect of insulin appears to be balanced by a vasoconstricting effect in healthy rodent models (Serne et al. 2006). Additionally, the vascular effect of insulin is observed to differ depending on a variety of factors such as; the metabolic health (and the species!) of the subject, the

section of the skeletal muscle vasculature studied and the dose and timing of the insulin stimulus (Clerk et al. 2004). Skeletal muscle glucose uptake preceeds the insulin-mediated increase in total muscle blood flow (Clark et al. 2003). This statement might appear to be incongruous with the hypothesis that the vascular and metabolic effects of insulin on skeletal muscle are coupled however, insulin-mediated capillary recruitment and glucose uptake are closely linked and reveal a direct relationship between insulin-mediated vasodilatation and glucose uptake which is not found when other vasodilators are used (Clark et al. 2003). Further evidence to support this relationship is provided by rodent models where reduced capillary recruitment is observed when insulin resistance is induced (Clark et al. 2003; Vincent et al. 2005).

It is suggested that insulin has a critical flow-mediated action, not on large calibre conduction and resistance vessels, but on terminal arterioles and the capillary beds (Sydow, Mondon, & Cooke 2005). Insulin is thought to have this effect through a NO-dependent action – further linking the metabolic interaction between insulin and the endothelium (Eringa et al. 2002). Insulin is recognised to activate endothelial Nitric Oxide Synthase (eNOS) by a signalling cascade which utilises IRS-1, PI3K and PKB – similar to the insulin signalling pathway involved in GLUT4 expression (Eringa et al. 2002; Sydow, Mondon, & Cooke 2005; Vincent et al. 2003). In animal models, genetic knockout of NO or pharmacological blockade of NOS activity both result in reduced skeletal muscle capillary recruitment and insulin resistance (Sydow et al. 2005; Vincent et al. 2003). In addition, human studies have shown that infusion of the eNOS inhibitor asymmetric dimethylarginine (ADMA), causes endothelial dysfunction and insulin resistance (Sydow et al. 2005) and circulating ADMA concentrations are higher in patients with T2D (Abbasi et al. 2001). Decreased NO bioavailability is considered to be the crucial factor in the aetiology of endothelial dysfunction (Imrie, Abbas, & Kearney 2010).

Interstitial insulin concentrations are more closely associated with skeletal muscle glucose uptake than plasma insulin concentrations, suggesting that insulin delivery to the myocyte (which requires capillary recruitment and trans-endothelial transport across the vascular endothelium) is a key determinant of the metabolic action of insulin (Castillo et al. 1994). Impaired insulin action in skeletal muscle capillary endothelium prevents capillary recruitment and trans-endothelial insulin transport and is mediated by reduced NO bioavailability which is a result of Akt/PKB interference leading to diminished eNOS activity (Barrett et al. 2009). Failure of skeletal muscle glucose uptake results in increased carbohydrate delivery to the liver which promotes lipogenesis and subsequent NEFA delivery to skeletal muscle, further increasing IMCL and myocyte insulin resistance by the mechanisms described in the previous section. The initial ‘uncoupling’ of insulin-stimulated NO production has been demonstrated by TNF α -mediated impairment of insulin-induced activation of Akt and eNOS and implicates the low-grade inflammatory process as a potentially founding factor in endothelial insulin resistance (Serne et al. 2006;Barrett et al. 2009;DeFronzo 2010).

1.8.4 Insulin resistance in liver

Carbohydrate and fat metabolism are highly influenced by circulating insulin, glucose and NEFA concentrations. In a state of chronic insulin resistance, accompanied by beta-cell dysfunction, NEFA stimulate hepatic glucose production through gluconeogenesis (Roden et al. 2000;Delarue & Magnan 2007). Under normal circumstances increased circulating NEFA does not increase hepatic glucose output due to down-regulation of hepatic glycogenolysis (hepatic autoregulation) however, in an insulin resistant state hepatic glucose production is increased by persisting glycogenolysis which may be mediated by increased circulating glucagon (Chen, Iqbal, & Boden 1999;Samuel et al. 2009) or may be mediated by chronically elevated concentrations of NEFA (Shah et al. 2002).

The failure of insulin to suppress hepatic glucose production is closely related to the degree of hepatic steatosis (Kotronen et al. 2007; Seppala-Lindroos et al. 2002). In addition, reduced hepatic insulin clearance is associated with hepatic steatosis (Kotronen et al. 2007). The source of fatty acids which lead to hepatic steatosis is debated, but is likely to be derived from chronic dietary excess and fatty acid release from visceral and/or subcutaneous adipose tissue. Hyperinsulinaemia, which is a compensatory response to peripheral insulin resistance, stimulates hepatic fatty acid synthesis from glucose and further exacerbates hepatic steatosis (Taylor 2008). In addition fatty acid oxidation is inhibited in the hepatocyte by the accumulation of malonyl CoA, which interferes with fatty acid transport into the mitochondria (Taylor 2008). Increased fatty acid oxidation in obese mice produces increased insulin sensitivity and decreased steatosis (Reid et al. 2008), and pioglitazone mediated stimulation of PPAR γ in patients with T2D reduces both hepatic glucose production and hepatic fat content (Ravikumar et al. 2008).

However, hepatic steatosis *per se* is not necessarily a mediator of hepatic insulin resistance. In mouse models, hepatic steatosis can be induced by pharmacological blockade of β -oxidation, without affecting insulin sensitivity (Grefhorst et al. 2005). Patients with familial hypobetalipoproteinaemia are unable to export VLDL from the liver and are susceptible to hepatic steatosis but do not display insulin resistance (Schonfeld et al. 2008). It is therefore possible that hepatic fat, like IMCL, is closely associated with insulin resistance but not a key mediator of its development. Thus, a combination of excess fatty acid delivery to the hepatocyte, increased intrahepatic fatty acid synthesis and hyperinsulinaemia produce a state hepatic insulin resistance and hepatic steatosis which, in turn promote hepatic glucose production and reduced insulin clearance.

Chapter 3 of this thesis aims to examine the differences in insulin sensitivity between sedentary pre-menopausal women with a family history of diabetes and matched controls. In

addition the relationship between adiposity, adipose tissue derived factors, lifestyle and substrate utilisation is explored in an attempt to understand the aetiology of insulin resistance.

1.9 Insulin resistance in obesity and lipodystrophy ('dysadiposity')

Obesity is strongly correlated with the development of insulin resistance and subsequent progression to T2D and premature cardiovascular disease (Barrett-Connor 1989; Ohlson et al. 1985; Ohlson et al. 1988; Wang et al. 2005). Section 1.9 illustrates the importance of fatty acid metabolism in the aetiology of tissue specific and total body insulin resistance. In addition, high risk groups such as South Asians appear to manifest greater sensitivity to the effects of excess adipose tissue, displaying dyslipidaemia and dysglycaemia at lower levels of adiposity than Caucasians (Razak et al. 2007; Whincup et al. 2005). Chandalia and colleagues report that South Asians have an expanded deep subcutaneous adipose pool and larger adipocytes than matched Caucasians (Chandalia et al. 2007). This may suggest that the increase in circulating inflammatory proteins (CRP, IL-6, PAI-1) found in South Asians is a consequence of a greater proportion of metabolically active adipose tissue, for a given BMI (Hall, Sattar, & Gill 2008). Increased body mass, even within 'normal' range body mass index (BMI) is associated with increase risk of T2D in a Japanese population (Nagaya et al. 2005). All adipose tissue is not however, metabolically equal. Offspring of patients with T2D tend to display increased visceral adiposity compared with subjects with no family history of T2D who were matched for total adiposity, in addition offspring subjects displayed greater insulin resistance which was closely associated with the degree of visceral adiposity (Nyholm et al. 1997; Nyholm et al. 2004). As in South Asians, Australian Aboriginals and Chinese populations, offspring subjects may display enhanced sensitivity to adiposity exhibiting lower insulin sensitivity for the same fat mass as found in matched controls (Kriketos et al. 2004).

Visceral adipose tissue is thought to have greater influence on insulin sensitivity than subcutaneous fat (Macor et al. 1997). Use of direct imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) have allowed distinction between visceral and subcutaneous adipose pools and are more sensitive methods of assessing these compartments than traditional anthropometry (Jia et al. 2003; Pi-Sunyer 2000; van der & Seidell 1993). An enlarged visceral adipose compartment may increase NEFA delivery to the liver via the splanchnic circulation (Basu et al. 2001), increase total circulating NEFA (Arner 2002) which may impact on skeletal muscle insulin resistance (Boden 1997; Corcoran, Lamon-Fava, & Fielding 2007) and cause pancreatic beta-cell toxicity (Taylor 2008; Tushuizen et al. 2007) and may be a source of pro-inflammatory adipokines (such as TNF α) which promote insulin resistance by direct action on insulin signalling and indirectly by inducing endothelial dysfunction (Montecucco, Steffens, & Mach 2008; Salmenniemi et al. 2005; Sydow, Mondon, & Cooke 2005).

Further support for visceral fat as an important metabolic contributor to insulin resistance is observed in patients with lipodystrophy. Lipodystrophy is a condition of disordered adipose tissue distribution and may be localised, partial or generalised. Lipodystrophy may be congenital or acquired and both partial and generalised conditions are associated with insulin resistance and dyslipidaemia (Hegele 2003; Monajemi et al. 2007). Congenital lipodystrophies are a heterogeneous group of syndromes which are associated with a variety of defects in insulin signalling, lipid synthesis, connective tissue substrate production and PPAR γ function (Monajemi et al. 2007). Acquired lipodystrophies are most commonly observed in Human Immunodeficiency Virus (HIV) sufferers who are treated with protease inhibitors (Carr et al. 2001; Garg 2004). The metabolic defects observed in lipodystrophy are frequently associated with reduced subcutaneous adipose tissue and increased visceral adipose tissue, although in some syndromes reduced peripheral subcutaneous adipose tissue is seen whilst truncal subcutaneous adiposity is preserved (Hegele 2003). Insulin resistance in these conditions is often severe (Beltrand et al. 2007), and is associated with reductions in

both leptin and adiponectin, as well as increased triglycerides, low HDL-cholesterol and in some syndromes, elevated circulating NEFA (Hegele 2003).

Many of these defects are common to the general insulin resistance state seen in pre-diabetic conditions such as the metabolic syndrome, impaired glucose tolerance, and in high risk groups such as South Asians and offspring of patients with T2D. Some lipodystrophic conditions have shown metabolic response to treatment with leptin (Beltrand et al. 2007; Park et al. 2008) and PPAR γ agonists (Maraldi et al. 2007). An improved metabolic phenotype in lipodystrophy is associated with increased subcutaneous adipose tissue mass (Hegele 2003; Monajemi et al. 2007), which emphasises the importance of adipose tissue site and function, rather than total adipose tissue mass, in the development of insulin resistance.

1.10 The effect of insulin resistance on people with a family history of T2D

First-degree relatives of patients with T2D are recognised to be insulin resistant (Humphriss et al. 1997; Perseghin et al. 1997) and also display increased clustering of cardiovascular risk factors (Micossi et al. 1987; Sarlund et al. 1992). In addition, these subjects also display pro-thrombotic tendencies with elevated levels of D-Dimer and Plasminogen Activator Inhibitor-1 (PAI-1) (Fernandez-Castaner et al. 1996; Mansfield, Stickland, & Grant 1997). Relatives may also exhibit an atherogenic lipoprotein profile, with elevated apolipoprotein B (apoB), and reduced levels of apolipoprotein A-1, with increased apoB in those with greater insulin resistance (Stewart et al. 1998). Further objective evidence of cardiovascular risk in this group was observed with respect to vascular function, where relatives displayed increased carotid intima-media thickness (Pannacciulli et al. 2003) and impaired endothelial function characterised by reduced bio-availability of nitric oxide (Caballero et al. 1999; Cersosimo & DeFronzo 2006; Goldfine et al. 2006).

1.11 Insulin resistance in women

Insulin resistance appears to have a greater impact on cardiovascular risk in women, both prior to and after the development of T2D (Becker et al. 2003; Hu et al. 2001; Hu et al. 2002). Tailored public health strategies have recently been suggested to tackle cardiovascular risk in women (Engberding & Wenger 2008; Evangelista & McLaughlin 2009). The presence of insulin resistance in women may be associated with other metabolic disturbances which are not present to the same extent in men, and which may confer increased risk (Regitz-Zagrosek, Lehmkuhl, & Weickert 2006). In insulin resistant subjects higher inflammatory markers have been reported in women compared to men, and remain significantly higher in women even after adjustment for BMI (Lakoski et al. 2006; Saltevo et al. 2008). This observation may simply reflect a greater amount of adipose tissue in women compared to men of a given BMI, although Saltevo and colleagues have also suggested that with increasing insulin resistance women display relatively greater reductions in adiponectin compared to men and that this may be indicative of increased adipose tissue macrophage infiltration (Saltevo, Kautiainen, & Vanhala 2009). The influence of circulating oestrogen in pre-menopausal women is recognised to have metabolic benefit; promoting fat oxidation, reduced inflammatory cytokines, reduced oxidative stress, reduced hepatic glucose output and subcutaneous, rather than visceral fat accumulation (Geer & Shen 2009). The development of insulin resistance in women is associated with attenuation of these protective mechanisms (Geer & Shen 2009). Polycystic ovarian syndrome (PCOS) is an insulin resistant state characterised by hyperandrogenism, visceral obesity, endothelial dysfunction and low grade inflammation (Giallauria et al. 2008a). It is therefore conceivable that the development of insulin resistance in women is mediated by different mechanisms to those observed in men and that low-grade inflammation may have greater influence on metabolic health in insulin resistant women compared to men. Therefore, chapter 3 of this thesis aims to specifically investigate mediators of insulin sensitivity in pre-menopausal women with, and without a family history of T2D.

1.12 Insulin resistance, cardiovascular risk and low grade inflammation

Adiposity is associated with higher circulating levels of pro-inflammatory cytokines such as c-reactive protein (CRP), interleukin-6 (IL-6), tumour necrosis factor- α (TNF α) and interleukin-1 (IL-1) (Montecucco et al. 2008). In addition, insulin resistance and obesity are associated with low levels of adiponectin which has anti-inflammatory activity (Folco et al. 2009). Increased circulating inflammatory cytokines may contribute to both the aetiology of insulin resistance, and the end-organ damage observed in the insulin resistant state (Festa et al. 2000; Kowalska et al. 2008). Therefore, low grade inflammation may be both a co-founder in the development of insulin resistance and co-conspirator in the metabolic dysfunction observed in this state. Elevated inflammatory factors are observed in obesity, however increased adipose tissue mass is also associated with increased macrophage recruitment to adipose tissue. Hormones such as leptin and adiponectin are produced by the adipocyte, IL-6 and TNF α are produced by both adipocytes and macrophages (Berg & Scherer 2005) and other inflammatory cytokines are likely to be derived from intra-adipose macrophages (Fain et al. 2004).

There is strong mechanistic evidence that TNF α is a major inflammatory protein and that it is directly involved in the pathogenesis of insulin resistance and atherosclerosis. Elevated plasma expression of TNF α is observed in T2D and atherosclerosis and is likely to be derived from adipose tissue (Berg & Scherer 2005). Interference with Akt/PKB activity has previously been outlined (Section 1.8.3) as a method by which TNF α induces endothelial dysfunction. Additional activation of the intracellular enzyme c-Jun N-terminal kinase (JNK) by TNF α contributes to endothelin-mediated vasoconstriction in the presence of insulin (Eringa et al. 2006). TNF α also activates NADPH oxidase (via PKC activation) resulting in superoxide generation which depletes NO, and through activated NF κ B produces cellular adhesion molecules (CAM) which stimulate leucocyte endothelial binding and further promote local inflammation and damage the endothelial barrier (Wagenmakers et al. 2006).

These factors result in NO deficiency and endothelin dominance producing both skeletal muscle insulin resistance (through reduced capillary recruitment) and increased peripheral vascular resistance (Serne et al. 2006; Pedersen 2006). This mechanism is complemented by the induction of lipolysis in adipose tissue (Moller 2000), exposing the vascular wall, skeletal muscle and other tissues to circulating NEFA (Pedersen 2006). Additionally, TNF α promotes the 'atherogenic lipid profile' characterised by reduced HDL and increased oxidised LDL, which increase plaque expansion (Popa et al. 2007). Blockade of TNF α using immunomodulators has had mixed results in human subjects, whilst reduction in inflammatory factors was achieved using specific agents, there have been less impressive improvements in insulin sensitivity (Dominguez et al. 2005).

The role of IL-6 in the pathogenesis of insulin resistance is controversial and recent appreciation of IL-6 as a 'myokine' reflects the complexity of the interaction between cytokines and their local and systemic effects (Pedersen 2006). Vettor and colleagues observed that IL-6 interferes with IRS-1 autophosphorylation and that plasma IL-6 concentrations correlate with the degree of insulin resistance (Vettor et al. 2005). However, plasma IL-6 concentrations also correlate with body mass and the relationship between IL-6 and insulin resistance may represent the more robust association between adipose tissue mass and insulin resistance (Jensen 2008). The effect of IL-6 is likely to depend on the target tissue studied. Visceral fat appears to 'overproduce' IL-6 (Jensen 2008), whereas in healthy individuals IL-6 stimulates both lipolysis and fat oxidation without net hypertriglyceridaemia (van et al. 2003). Contraction of skeletal muscle stimulates IL-6 production by the myocyte, which stimulates fatty acid oxidation by activation of adenosine monophosphate kinase (AMPK) (Pedersen 2006).

TNF α , IL-1 and CRP may promote further inflammatory and pro-coagulatory protein production (i.e. ICAM/VCAMs) through stimulation of NF κ B (Sarada et al. 2008), and these

effects are enhanced in the presence of increased glucose (Iwasaki et al. 2007; Wilson, Ryan, & Boyle 2006). CRP correlates with insulin sensitivity in non-diabetic women, and may predict the development of T2D (Pradhan et al. 2001; Pradhan et al. 2003), however it is unclear whether CRP has a direct physiological role in insulin resistance, or whether it is merely a marker of inflammatory activity and the underlying pathogenic processes (Sattar & Lowe 2006; Wilson, Ryan, & Boyle 2006).

Normoglycaemic first-degree relatives of patients with T2D display evidence of a pro-inflammatory state (Tesauro et al. 2007), and evidence of insulin resistance and impaired vascular function (McEleavy et al. 2004; Scuteri et al. 2008). However, McEleavy and colleagues and Scuteri and colleagues used carotid-radial pulse wave velocity and flow mediated brachial artery dilatation respectively. These techniques are considered to be surrogates for endothelial function but principally measure vascular function in conduction vessels. Therefore these techniques demonstrate impaired large vessel reactivity, but are not designed to examine microvascular function, particularly skeletal muscle capillary recruitment. To date, the author is not aware of any studies on skeletal muscle capillary recruitment in normoglycaemic offspring of patients with T2D.

Kriketos and colleagues (2004) did not demonstrate increased inflammation in normoglycaemic first-degree relatives, however this study compared groups who were well matched for adiposity, whereas Scuteri and co-workers (2008) studied relatives who had greater adiposity than the comparator control group (Scuteri et al. 2008; Kriketos et al. 2008). Non-obese women with polycystic ovarian syndrome did not show increased evidence of inflammation compared to controls, despite displaying significantly lower insulin sensitivity (Martinez-Garcia et al. 2009). It seems likely that inflammation contributes to the pathogenesis of both insulin resistance and atherosclerosis (Vettor et al 2005; Jeppesen et al. 2008), but that measurable increases in inflammation may only be apparent with increased

adiposity or detectable change in insulin sensitivity (Ruotsalainen et al. 2006; Ruotsalainen et al. 2008). It is also conceivable that systemic measurements of inflammation are too crude to detect the interaction between low grade inflammation and skeletal muscle capillary endothelial dysfunction which are likely to be present early in the pathogenesis of insulin resistance (Bakker et al. 2009).

1.13 Adipose tissue hormones and insulin resistance

Adipose tissue hormones also contribute to insulin sensitivity, however their physiological effects differ between states of metabolic health and disease (Stefanyk & Dyck 2010). Plasma leptin concentrations correlate directly with adipose tissue mass (Fruhbeck, Jebb, & Prentice 1998) and obese individuals with high circulating leptin are insulin resistant (Considine et al. 1996; Weyer et al. 2001). Plasma adiponectin concentration is inversely related to adipose tissue mass (Arita et al. 1999) and circulating adiponectin concentrations are lower in patients with obesity and T2D (Hotta et al. 2000; Weyer et al. 2001). In pre-diabetic states adiponectin is reduced (Lihn et al. 2003; Kowalska et al. 2008), whilst high circulating adiponectin is associated with reduced inflammatory markers and reduced risk of T2D (Duncan et al. 2004). In first-degree relatives of patients with T2D circulating leptin is frequently increased, however these increases are more strongly related to adiposity and insulin resistance than family history and are indicative of the underlying mechanisms which increase risk of T2D in this group (Nyholm et al. 1997; Vauhkonen et al. 1998). Initial speculation on the role of adipokines in insulin resistance was based on these observations and was predicated on the assumption that the development of obesity and insulin resistance was ‘caused’ by hyperleptinaemia and/or hypoadiponectinaemia. However, reversal of leptin deficiency in both rodents (Larcher et al. 2001) and humans (Beltrand et al. 2007) improves insulin sensitivity. Therefore recent evidence would suggest that the relationship between adiponectin and leptin in metabolic health is one of synergistic promotion of insulin

sensitivity and in states of insulin resistance and obesity, that this synergy is lost (Dyck 2009).

In lean insulin sensitive subjects both leptin and adiponectin stimulate skeletal muscle fatty acid oxidation (Kieffer & Habener 2000; Margetic et al. 2002). Leptin stimulates fatty acid partition towards oxidation and through phosphorylation of ACC2 prevents accumulation of malonyl CoA (Dyck 2009). These actions prevent accumulation of DAG and ceramides, maintaining insulin sensitivity and are mediated by direct activation of AMPK (Minokoshi et al. 2002; Steinberg & Jorgensen 2007) (Havel 2004). Leptin may also reduce expression of fatty acid transporters (CD36) which could contribute to reduced IMCL however this effect has, to date, only been observed in rodents (Dyck 2009). Leptin also has central actions, acting as an energy balance hormone, triggering hypothalamic satiety (Vettor et al. 2005) and stimulating skeletal muscle AMPK by α -adrenergic activity (Minokoshi et al. 2002).

In healthy individuals adiponectin also stimulates fatty acid oxidation in skeletal muscle through AMPK activation by the adiponectin receptor 1 (AdipoR1) and may directly inactivate ACC2 (Dyck 2009; Steinberg & Jorgensen 2007). Adiponectin also stimulates fatty acid oxidation in the liver, reduces *de novo* lipogenesis and decreases both hepatic glucose output and circulating NEFA and VLDL (Tsochatzis, Papatheodoridis, & Archimandritis 2009). These effects are associated with improved total body insulin sensitivity (Furler et al. 2006).

Elevated concentrations of circulating leptin are found in obesity in humans and in states of high-fat feeding in rodents (Stefanyk & Dyck 2010). In these conditions, leptin is unable to stimulate skeletal muscle fat oxidation (Steinberg et al. 2002) but since administration of the AMP analogue 5-aminoimidazole-4-carboxamide-1- β -D-ribose (AICAR) fully

activates AMPK in this situation it is suggested that the defect in obesity is an impairment of leptin-mediated AMPK activation (Dyck 2009; Steinberg & Jorgensen 2007). Knockout mice, high-fat fed rodents and obese humans display increased levels of the suppressor of cytokine signalling 3 (SOCS3) protein (in skeletal muscle) which appears to interfere with stimulation of AMPK by inhibiting leptin signalling (Steinberg et al. 2006). In addition to the reduction in adiponectin concentration in obesity and insulin resistance, skeletal muscle also appears to be unable to promote fatty acid oxidation in response to adiponectin exposure (Mullen et al. 2009). Acute saturated fat feeding provokes reductions in skeletal muscle fatty acid oxidation and ACC2 phosphorylation despite adiponectin exposure and is followed by chronic (4 weeks) increased expression of fatty acid transporters and myocyte DAG and ceramide content (Mullen et al. 2009). This is unlikely to be related to changes in adiponectin receptor expression (Steinberg & Jorgensen. 2007; Mullen et al. 2009). However, longer term obesity and insulin resistance may reduce receptor expression. In cultured myotubes derived from obese, diabetic or formerly-obese subjects an inability to increase adiponectin receptor expression after exposure to leptin and adiponectin was observed (McAinch et al. 2006). Finally, adiponectin deficiency may contribute to the low grade inflammatory state in obesity since reduced adiponectin receptor expression in adipose tissue results in increased expression of the macrophage attracting chemokine monocyte chemoattractant protein-1 (MCP1) (Yamauchi & Kadowaki 2008).

In health, leptin and adiponectin appear to exert a complementary effect upon skeletal muscle fat oxidation, which promotes insulin sensitivity. However in obesity and insulin resistance they are unable to maintain these processes despite an intact downstream mechanism (AMPK). In fact the development of obesity would seem to induce skeletal muscle resistance to these hormones and chronic supraphysiological concentrations of leptin may downregulate adiponectin receptor expression resulting in a vicious circle of impaired skeletal muscle fat oxidation, insulin resistance and adipose tissue inflammation (Fang et al. 2009; Stefanyk & Dyck 2010; Yamauchi & Kadowaki 2008). Therefore one of the aims of this thesis is to

examine the interaction between adiposity, adipokines and inflammation with respect to their related and independent effects on insulin sensitivity.

1.14 Genetic predisposition to insulin resistance

The previous sections of this chapter have outlined a number of metabolic abnormalities which contribute to the development of insulin resistance and subsequently increase the risk of T2D and premature cardiovascular disease. Evidence for a gene-environment interaction has been observed in specific ethnic groups (Pimas, Oji-Cree, Torres-Strait Islanders etc.) and this hypothesis has been generalised to more genetically diverse populations such as South Asians and offspring of people with T2D.

The search for the genetic origin of T2D has focused on potential ‘diabetogenic’ gene polymorphisms, clustering of specific polymorphisms in high-risk populations and mechanisms of inheritance. Paradigms for the single gene predisposition to diabetes exist in patients with Maturity Onset Diabetes of the Young (MODY). These patients display a strong family history of diabetes at an early age and have frequently been misdiagnosed as type 1 diabetes due to the age of onset. MODY patients have a monogenic diabetes which has a variable treatment and natural history depending upon the genetic mutation, in most cases however, insulin resistance is not a key component of the pathophysiology (Olek 2006). The prevalence of MODY is very low, affecting approximately 0.17 per 100,000 children in the UK (Ehtisham et al. 2004).

Other forms of inherited diabetes are observed in maternally transmitted mitochondrial DNA (mtDNA) mutations. These patients suffer from T2D but also from a host of other mitochondrial diseases, such as deafness, myopathy, lactic acidosis and encephalopathy and are therefore distinct from ‘common’ T2D (McIntyre & Walker 2002). Rare monogenic

forms of insulin resistance are seen in inherited forms of lipodystrophy (Dunnigan-type) (Hegele 2003) and Type A insulin resistance syndrome (Young et al. 2005). These conditions are clinically striking and have a markedly different phenotype when compared with T2D (Jiang et al. 2006).

In view of the insidious onset of T2D, accompanied by a gradual change in phenotype it is clear that significant monogenic abnormalities are not involved in the majority of cases of T2D. Instead T2D has a polygenic basis with more subtle alterations in gene function which appear to require specific environmental conditions to be prevalent to allow the manifestation of the disease. Single-nucleotide polymorphisms (SNPs) for the leptin receptor are associated with increased risk of T2D in people from Northern China (Qu et al. 2008). Abnormalities near a fatty acid binding protein on chromosome 4 are associated with insulin resistance in Pima Indians and Europeans with T2D (Humphreys et al. 1994). Variations in a gene which codes for a calcium sensing protein may have a minor effect on insulin action in Pima Indians (Ma et al. 2008). SNPs for the PPAR α gene are associated with increased risk of progression from IGT to T2D (Andrulionyte et al. 2007). Combinations of SNPs for the Calpain-10 protease (which may have a role in insulin signalling (Paul et al. 2003)) appears to increase risk of T2D, especially when combined with other local SNPs (Malecki 2005;McIntyre & Walker 2002). Multiple genetic abnormalities in insulin signalling, insulin secretion, fatty acid uptake and intracellular substrate utilisation have been determined (McIntyre & Walker 2002). These observations support the hypothesis that type 2 diabetes arises from a complex interaction between fatty acid metabolism, skeletal muscle substrate utilisation, hepatic autoregulation and both insulin resistance and reduced secretion. In addition the roles of adipose tissue hormones and cytokines would appear to modulate the hepatic, skeletal muscle and adipose tissue responses to fatty acids, insulin and glucose and in situations of chronic energy excess, contribute further to the progression towards T2D.

1.15 Environmental impact on genetic predisposition

The comparison between rates of diabetes in genetically identical groups who are exposed to different environments is compelling evidence for the role of environment in the aetiology of diabetes. This is most striking in comparison between American and Mexican Pima Indians (Valencia et al. 1999), but is also apparent in other groups (e.g Torres Strait Islanders, Oji Cree Indians) who have been exposed to a rapid change in their environment (Hegele et al. 2003; Longstreet et al. 2007). The high prevalence of diabetes in the developed world and expected increase in the prevalence of the disease in the Indian sub-continent and China (Wild et al. 2004) provides evidence that the influence of a sedentary lifestyle and an energy dense diet with high quantities of fat and refined carbohydrate is a powerful environmental influence at a population level. This is likely to be related to increased obesity, but there may also be an independent contribution from diet (Ventura et al. 2009) and physical activity (Balkau et al. 2008). Ethnic groups who have migrated from cultures where lifestyles are traditionally very active and consume diets which are high in fibre and low in fat, to a Westernised lifestyle often display a rapid increase in prevalence of T2D compared with their parent populations (Nakanishi et al. 2004). People with a parent with T2D who are sedentary are insulin resistant and display increased risk of diabetes, but in offspring who are active, the tendency towards metabolic dysfunction appears to be ameliorated (Gill & Malkova 2006).

Environmental influences are likely to begin at an early stage in human development and appear to be able to induce physiological changes which predispose to a variety of chronic diseases. Osmond and Barker (2000) proposed that poor fetal growth predisposed to later development of hypertension, coronary heart disease, insulin resistance and T2D (Osmond & Barker 2000). It is suggested that fetal undernutrition ‘programmes’ the fetus to protect cerebral development by ensuring adequate perfusion and nutrition, at the expense of the development of other viscera and the fetus as a whole (Osmond & Barker 2000). With respect to the later development of T2D, it is recognised that small babies display insulin resistance

in later life and in some ethnic groups (e.g. South Asians) this is manifest as dysglycaemia in childhood (Yajnik et al. 1995). Protection of the developing fetal brain results in a catabolic state *in utero*, with a metabolism which is glucose-sparing to allow adequate substrate delivery to the brain. This glucose-sparing is achieved by the development of peripheral insulin resistance, evidenced by low rates of glycolysis and glycolytic ATP production during exercise in people who were small babies (Taylor et al. 1995). A catabolic fetal state is proposed to result in reduced circulating insulin, and increased glucocorticoids, this would produce a metabolic shift towards protein and fat metabolism for energy production and may explain low subcutaneous fat and muscle bulk in low birth weight babies (Bjorntorp 1995; Robinson et al. 1991). Visceral 'sacrifice' may lead to lower beta-cell mass and subsequently a tendency towards insulin deficiency (Osmond & Barker 2000).

Small baby fetal programming results in early metabolic changes which adapt to an environment of 'thrift'. The plasticity of the developing fetus allows these influences to affect organogenesis and physiological function to a greater extent than at any other time in life and because this occurs at a critical developmental period these changes are subsequently 'locked in'. The risk of developing T2D is increased in small babies who display rapid 'catch-up' growth, particularly after the age of 2 (Barker 2005). It is suggested that the rapid increase in weight after the age of 2 is characterised by a disproportionate increase in fat mass relative to muscle mass, which would be expected to result in insulin resistance (Eriksson et al. 2002).

Fetal macrosomia is more common in women with gestational diabetes or T2D, and is also associated with subsequent risk of T2D in the offspring (McCance et al. 1994). Birthweight would appear to have a 'U-shaped' relationship to the development of T2D, and appears to be increased by both low and high birthweight, particularly when there is increased adiposity after the age of 2 years (Barker 2005). These findings would appear to be contradictory, however it would seem likely that there are multiple routes towards T2D, as might be

expected given the complex nature of the metabolic dysfunction. Those with low birthweight and accelerated catch-up growth can be considered to be programmed towards an environment of thrift by their intrauterine environment and are subsequently unable to alter their insulin resistant 'glucose-sparing' metabolism in the presence of increased nutrient supply. Those with high birthweight are more likely to be born to diabetic mothers, and therefore display genetic predisposition towards diabetes (McCance et al. 1994), however it may be suggested that this could also be an effect of intrauterine programming where the fetus is exposed to high circulating insulin, carbohydrate and NEFA levels and generating an obese insulin resistant baby (Dabelea et al. 2000). Hattersley and Tooke have suggested that the observed 'small baby' phenotype could also be due to a genetically mediated insulin resistance, which prevents adequate growth *in utero* (Hattersley & Tooke 1999). In support of Hattersley and Tooke's 'fetal insulin hypothesis' Lindsay and colleagues (2000) observed that low fetal birth weight may be mediated by paternal diabetes, suggesting that low birth weight is genetically influenced since paternal physiology has no effect on intrauterine environment (Lindsay et al. 2000). Embryo transfer studies in genetically normoglycaemic and hyperglycaemic rats also suggests that a normal intrauterine environment cannot overcome the genetic tendency towards diabetes, but that exposure of genetically euglycaemic rats to a hyperglycaemic intrauterine environment promotes diabetes in later life (Gill-Randall et al. 2004). Additionally, recent analysis of T2D-promoting SNPs in mothers and offspring has shown that fetal inheritance of specific pro-diabetes SNPs are associated with low birthweight, which supports the fetal insulin hypothesis (Freathy et al. 2009).

In summary, T2D is increased in those with a genetic predisposition and in those who are subjected to intrauterine undernutrition (Barker et al. 2009; Lindsay et al. 2000). The risk may be augmented by increased weight gain after the age of two, in both small and large babies (Barker 2005). However early 'catch-up' growth may be a physiological compensation for poor intra-uterine growth and not pathogenic (Beltrand et al. 2009). Intrauterine programming may occur in small and large babies, predisposing to obesity, insulin resistance

and other chronic diseases and is likely to be mediated by both genetic and environmental influences (Freeman 2009; Lindsay 2008). Individuals with a positive family history of diabetes are more susceptible to the development of metabolic dysfunction related to a sedentary lifestyle and display relative insulin resistance and dyslipidaemia at normal body weights (Gill & Malkova 2006; Guerrero-Romero & Rodriguez-Moran 2006).

1.16 Physical activity and diabetes risk

Lifestyle interventions in people with impaired glucose tolerance (IGT) have been proven to reduce or delay progression to T2D (Knowler et al. 2002; Pan et al. 1997; Tuomilehto et al. 2001). In each of these studies lifestyle intervention consisted of intensive dietary modification as well as increased physical activity and goals included weight loss targets, although the Da Qing study did show reduced incidence of diabetes in an ‘exercise-only’ group who did not reduce weight (Pan et al. 1997). In addition, lifestyle intervention successfully prevents T2D in other groups at increased risk of the condition: specifically, obese men (Davey et al. 2005) and South Asians (Ramachandran et al. 2006).

Early epidemiological studies of environmental influences on diabetes risk observed a reduced risk of diabetes in non-diabetics who participated in regular exercise, in addition this reduction in risk appeared to be greater in those who participated in more frequent exercise (Hu et al. 1999; Manson et al. 1992; Paffenbarger et al. 1997). Although diabetes is associated with obesity and high physical activity levels are associated with lean body morphology, the benefit of exercise in preventing T2D persists even in obese patients (Hu et al. 2004) and may have greater metabolic effects in those at greatest risk of diabetes (Gill & Cooper 2008; Helmrigh et al. 1991; Lynch et al. 1996).

Exercise may reduce risks of both diabetes and cardiovascular disease by mediating multiple metabolic changes. However, it should be appreciated that exercise and physical activity do not necessarily mean the same thing. 'Physical activity' may refer to the amount of habitual movement made during a usual day, whilst 'exercise' may be interpreted as a volitional attempt to engage in an undertaking which is specifically designed to raise activity for a period above that of habitual levels. When examining the impact of exercise interventions upon insulin sensitivity and obesity, it is therefore important to appreciate the type, duration, frequency and workload of the intervention and the pre-intervention characteristics of the studied population. With this in mind, the following sections examine the effects of an acute single exercise session, an exercise training intervention and the mechanisms by which these interventions affect metabolic health, as a background to Chapters 4 and 5. In addition, the methods by which exercise induces reduction in body mass and fat mass specifically are examined to provide background to Chapter 6.

1.16.1 Metabolic impact of a single exercise session

Acute effects of exercise can be observed in a number of metabolic pathways, even in response to a single session of exercise. Consistent reductions in triglycerides and increases in HDL-cholesterol have been observed at 12-48 hours after a single session and the degree of benefit is likely to be related to the energy deficit of the session (Thompson et al. 2001). Additionally, reduced blood pressure is present for 12-16 hours after an exercise session (Thompson et al. 2001).

Exercise mediates improves skeletal muscle glucose uptake through a final common pathway of increased GLUT4 expression and it is recognised that this is not achieved through acute activation of the insulin signalling pathway (Holloszy 2005). Instead, skeletal muscle contraction may stimulate insulin-independent translocation of GLUT4 by release of calcium

from the sarcoplasmic reticulum and subsequent activation of calmodulin-dependent protein kinase (CaMK) (Wright et al. 2005). Glucose delivery to skeletal muscle is also enhanced by increased capillary recruitment (Wojtaszewski & Richter 2006). Contraction may stimulate muscle glucose uptake by other mechanisms. AMPK activation can promote insulin-independent glucose uptake, but it is unclear if contraction can independently activate AMPK to a significant degree (Bosselaar, Smits, & Tack 2009; Wojtaszewski & Richter 2006). These changes describe an acute increase in skeletal muscle glucose uptake, rather than in insulin sensitivity. Therefore, these effects are related to increased insulin-independent membrane expression of intramyocellular GLUT4 from established storage pools. These mechanisms may partially account for the acute exercise-mediated increase in glucose uptake by skeletal muscle, however they do not persist beyond approximately three hours after exercise (Young et al. 1987). Subsequent increases in acute post-exercise insulin sensitivity persist beyond the initial period and are thought to be related to separate mechanisms.

After the initial increase in contraction-related glucose uptake, other mechanisms contribute to continued stimulation of GLUT4 translocation. These mechanisms appear to be related to increased activity of components of the insulin signalling cascade. At a period of approximately 4 hours after a single exercise session, increased insulin receptor substrate-2 (IRS-2) activity results in production of phosphatidylinositol-3,4,5-triphosphate (PIP₃) by stimulation of PI3K (Howlett et al. 2006). PIP₃ promotes atypical PKC function which is associated with increased insulin-mediated translocation of GLUT4 and may be implicated in exercise-mediated translocation and improved insulin sensitivity in the post-exercise state (Wojtaszewski & Richter 2006). Additionally, since AMPK is considered to be sensitive to the energy state of the myocyte it has been suggested that glycogen depletion may stimulate AMPK to directly influence glucose uptake (Borghouts & Keizer 2000; Jensen, Wojtaszewski, & Richter 2009).

Perseghin and colleagues (1996) described increased insulin sensitivity, intramuscular glucose-6-phosphate concentrations and glycogen synthesis in offspring of patients with T2D in response to a single exercise session and further increases after a six-week exercise intervention (Perseghin et al. 1996). AMPK has binding affinity for both glycogen and the enzyme glycogen synthase and McBride and Hardie (2009) have hypothesised that AMPK stimulates glucose uptake and suppresses glycogen synthesis during exercise as glycogen stores deplete (McBride & Hardie 2009). The exact mechanisms by which AMPK activation may increase direct glucose uptake in the acute post-exercise period are currently incompletely understood but may, in part, be mediated through Akt effects on GLUT4 vesicle exocytosis (Geiger et al. 2006; Jensen, Wojtaszewski, & Richter 2009). Myocellular energy deficit may be manifest by elevated levels of AMP or depletion of ATP or glycogen. AMPK has a well recognised role as a ‘guardian of cell energy’ and responds to relative energy deficit by inhibiting ACC2 which reduces concentrations of malonyl CoA, subsequently stimulating fatty acid oxidation (Jensen, Wojtaszewski, & Richter 2009; Turcotte & Fisher 2008). As previously described, reduced intramyocellular DAG and ceramide improve insulin signalling (Section 1.8.2). Exercise may induce activity of the p38 mitogen-activation protein kinases (MAPK) which can increase the activation of translocated GLUT4, and subsequently act in concert with insulin to promote further glucose uptake (Geiger et al. 2005). However, other authors have suggested that there is little evidence for an effect on the proximal components of the insulin-signalling cascade in increased exercise-mediated insulin sensitivity (Wojtaszewski et al. 2000). Instead, it is suggested that the GLUT4 pools which have recently recycled from the cell membrane after the initial contraction/hypoxia related stimulus remain more sensitive to insulin stimulation than prior to exercise (Geiger et al. 2006; Holloszy 2005). Although the mechanisms are incompletely understood there is a consistent observation that a single acute exercise session can increase insulin sensitivity and a recent review of this subject suggested that even given the wide variety of durations, intensities, subjects and types of exercise previously studied, a single session can induce improvements in insulin sensitivity for up to forty-eight hours (Turcotte & Fisher 2008).

1.16.2 Metabolic impact of repeated exercise sessions

The acute effects of a single exercise session do not persist and appear to be at least partly dependent upon the energy status of the myocyte, with reversal of exercise-mediated insulin sensitivity by glycogen repletion (Young et al. 1983). However, after an endurance training programme elderly women displayed persisting increases in insulin sensitivity despite a 72-hour interval between testing and the final exercise session (DiPietro et al. 2006). Younger but not older women display prolonged improvements in insulin sensitivity and this may be attributable to chronic adaptations in response to exercise (Goulet et al. 2005). Adaptive changes in skeletal muscle are at least partly responsible for improved insulin sensitivity outwith the 'acute' period. Although the precise changes in insulin signalling in response to exercise remain controversial (Christ-Roberts et al. 2004; Hawley & Lessard 2008), it is likely that chronic increased expression (as well as acute translocation) of GLUT4 is involved (Henriksen 2002). There is some evidence that exercise training can increase insulin receptor, Akt, GLUT4 and glycogen synthase levels and that increased PI3K signalling may occur also however, the significance of these findings in the greater context of muscle insulin sensitivity is unclear (Wojtaszewski & Richter 2006).

Repeated exercise sessions may stimulate other adaptive changes which can contribute to both improved insulin sensitivity and metabolic health. Increased oxidative capacity and capillary density were observed in skeletal muscle in response to aerobic exercise (Dube et al. 2008). In addition, increased adipose tissue insulin sensitivity has been observed 72-hours after completion of a 6-week exercise intervention (Shojaee-Moradie et al. 2006). The latter occurred in the absence of reduced body mass or fat mass but was associated with reduced visceral adipose tissue (Shojaee-Moradie et al. 2006). Other metabolic changes which occur after the 'acute' post-exercise period may also influence insulin sensitivity, for example: reduced circulating leptin has been observed in response to the energy deficit of an exercise intervention, but only after a 48-hour delay (Essig et al. 2000). Improved endothelial function

has been observed in response to acute exercise, but this effect is augmented by further bouts of exercise and persists for approximately one week (Haram et al. 2006). Increased AMPK activity may be associated with acute post-exercise increases in insulin sensitivity but repeated exercise sessions induce upregulation of AMPK and this may be partly responsible for skeletal muscle adaptation to exercise (Viollet et al. 2009).

1.16.3 Other insulin-sensitising effects of exercise

Exercise also mediates improved insulin sensitivity through a number of different mechanisms. Although total body weight may not change, body morphology may alter with increased lean tissue, reduced total body fat and reduction in specific adipose tissue compartments (Bouchard et al. 1990). Exercise may preferentially reduce central adiposity (Bo et al. 2008) and particularly visceral adipose tissue, producing different metabolic effects in comparison to a global reduction in total body fat (Johnson et al. 2009; Miyatake et al. 2002). Exercise also reduces markers of low grade inflammation in insulin resistant conditions and does so without significant weight or fat loss (Kadoglou et al. 2007; Petersen & Pedersen 2005). Additionally, exercise induces increased fat oxidation without reduction in adiposity (Venables & Jeukendrup 2008). Exercise mediated reduction of fat mass is associated with reduced circulating leptin (O'Leary et al. 2006) and increased fat oxidation (Goodpaster, Katsiaras, & Kelley 2003). In addition, circulating leptin concentrations after an exercise intervention correlated with fat oxidation in obese subjects, suggesting that increased fat oxidation and insulin sensitivity are partially mediated by improved leptin sensitivity in response to exercise (Solomon et al. 2008).

Further evidence that fat loss *per se* is less important than reduction in metabolically active adipose (such as visceral fat, or deep subcutaneous fat) or improvement in adipose tissue function can be observed in studies of obese patients who undergo fat reduction surgery.

Large volume liposuction has been shown to produce impressive cosmetic reduction in central adiposity, but does not alter visceral fat or other risk factors for T2D or coronary heart disease (Klein et al. 2004; Mohammed et al. 2008). Klein and colleagues (2004) report reduced circulating leptin after removal of large volumes of adipose but this is not associated with evidence of improved leptin, or insulin, sensitivity (Klein et al. 2004). Leptin is recognised to correlate with fat mass, however reduction of fat mass by exercise or dietary methods is likely to alter fat composition and function differently from surgical intervention (Mohammed et al. 2008). Ybarra and co-workers observed alterations in NEFA and lipids after liposuction, but not insulin sensitivity (Ybarra et al. 2008).

In summary, exercise improves insulin sensitivity after an acute session by mechanisms of insulin-independent and insulin-dependent stimulation of GLUT4 cycling. These mechanisms of action are multiply redundant but are probably related to both the physical contraction of the myocyte and the acute energy deficit (especially glycogen depletion) of the cell (Eriksen et al. 2007; Holloszy 2005). Further acute metabolic benefits are recognised with respect to blood pressure and lipids (Thompson et al. 2005). Chronic adaptive responses to exercise are also seen and with respect to increased peripheral insulin sensitivity, are likely to be related to increased oxidative capacity, skeletal muscle vascularity and upregulation of the pathways of both GLUT4 expression and insulin signalling (Colberg & Grieco 2009; Dube et al. 2008). These metabolic changes are accompanied by improved endothelial function and reduction in low grade inflammation although the variety in study design and associated results make analysis of the chronic effects of exercise independent of the effects of reduced body mass or adiposity difficult to determine (Esposito et al. 2003; Petersen & Pedersen 2005). However, it is likely that exercise interventions also improve insulin sensitivity in ‘at risk’ groups through reduction of body fat and in particular, visceral adipose. In addition independent of fat loss, altered adipose tissue function appears to contribute to improved insulin sensitivity by increasing lipid oxidation and restoring leptin sensitivity and is likely to contribute to a reduction in the chronic inflammatory state. Chapter 4 of this thesis will explore the acute

metabolic effects of exercise, by studying subjects within twenty four hours of an exercise session with repeat assessment after forty eight hours of sedentary activity, with the aim of detecting persisting metabolic adaptations to repeated exercise and defining those which are a consequence of a single bout of activity.

1.16.4 Exercise in persons at risk of type 2 diabetes

Exercise may have amplified metabolic effects in those who are at greater risk of the development of T2D. Post-prandial hyperinsulinaemia was reduced to a greater extent in obese insulin resistant men compared to lean insulin sensitive men in response to a pre-prandial exercise session (Gill et al. 2004). The magnitude of the exercise-mediated improvement in insulin sensitivity in the HERITAGE family study was related to baseline insulin resistance (An et al. 2005). Studies of physical activity and insulin sensitivity in offspring of people with T2D have observed that the increased insulin resistance associated with a family history of T2D is partially (Ahn et al. 2004) or completely (Higgins et al. 2005) ameliorated by high levels of habitual activity. Similarly in low-birthweight adult males, the tendency to hyperinsulinaemia and the metabolic syndrome were attenuated by regular participation in vigorous physical activity (Laaksonen et al. 2003). By careful matching for age and body mass and by applying recruitment criteria specifying less than one hour of planned physical activity per week, the studies described in chapters 3-5 aim to detect baseline and post-intervention differences in metabolic and physical characteristics which are determined largely by the presence, or absence of a parental history of T2D.

1.17 Lifestyle interventions for weight loss

Weight loss is a desired outcome for many individuals (particularly women) who are overweight, whether they are motivated to do so for cosmetic, psychological or medical reasons (Green et al. 1997). Individual dietary counselling is effective in reducing body mass

and is associated with reduced incidence of diabetes and improved blood pressure control (Avenell et al. 2004). However dietary weight loss is frequently unsuccessful and there are a number of psychosocial factors which limit an individual's ability to adhere to restriction of energy intake (Thomas et al. 2008). The addition of a physical activity intervention to increase energy expenditure in combination with reduction of energy intake results in greater weight loss and reduced weight regain (Steyn, Lambert, & Tabana 2009). The major determinant of fat loss in response to a lifestyle intervention is the energy deficit incurred (Strasser, Spreitzer, & Haber 2007) however, there is marked inter-individual variation in the extent of weight and fat loss in response to an exercise intervention (Byrne et al. 2006; King et al. 2008; Snyder et al. 1997). Predicted reductions in weight or fat mass are dependent upon the individual achieving the energy deficit calculated by either dietary restriction (Vogels & Westerterp-Plantenga 2007), increased physical activity (Byrne et al. 2006; Colley et al. 2008; McTiernan et al. 2007), or a combination of both. The observed variation between actual and predicted weight loss in response to an exercise intervention has previously been attributed to volitional or subconscious behavioural change such as increased energy intake (King et al. 2007), alteration in dietary composition (Finlayson et al. 2009) and decreased habitual physical activity or 'Non-Exercise Activity Thermogenesis' (NEAT) (Levine et al. 1999; Wang et al. 2008). However, recently identified variations in the FTO gene have suggested that individuals with these genetic variants are predisposed to overweight and obesity (Frayling et al. 2007) and it is possible that similar 'innate' factors could predispose to suboptimal weight loss response to a negative energy balance.

1.17.1 Diet and weight loss

The use of a hypocaloric diet to invoke energy deficit has been shown to reduce total body mass, fat mass and fat-free mass (Durrant et al. 1980). Low calorie, low carbohydrate, very low calorie and low fat diets are effective in inducing short-term weight reduction but are limited by patient tolerance and compliance and may not be associated with long-term weight maintenance (Strychar 2006). Very low calorie diets have the greatest reduction in energy

intake and are therefore associated with the greatest weight loss (~ 30% of body weight at 1 year) but have risks related to electrolyte abnormalities and malnutrition (Strychar 2006). For this reason these diets require close medical supervision. Dietary intervention appears to be an effective weight loss strategy in all groups; children (Shalitin et al. 2009), adults (Abete et al. 2009), the elderly (Houston, Nicklas, & Zizza 2009), both sexes (Al et al. 2009; Dengel et al. 1994) and patients with insulin resistance (Dyson 2008). However, restriction of energy intake is associated with reduced muscle mass which may be deleterious to both functional capacity and total body insulin sensitivity, particularly in the elderly (Frimel, Sinacore, & Villareal 2008; Kelley et al. 1999).

In addition to the physical reduction in body mass, dietary induced weight loss has additional metabolic benefits. A 6.1 kg reduction in body mass after a 3-month dietary intervention was associated with reduced liver fat and increased hepatic glucose uptake (Sato et al. 2007). Low carbohydrate diets have been shown to decrease body mass, markers of hepatic steatosis and increase insulin sensitivity (Ryan et al. 2007). Hypocaloric diet induced a 6.7 kg reduction in body mass over 12 weeks and was associated with increased insulin sensitivity, improved lipid profile and both total, and multimeric circulating adiponectin (Polak et al. 2007). Raatz and co-workers (2005) did not observe a metabolic or weight loss benefit of different diet compositions beyond that observed by the energy deficit incurred (Raatz et al. 2005). However, recent results from Volek and colleagues (2009) suggest that carbohydrate restriction may have greater impact upon lipids, insulin sensitivity, weight loss and regional adiposity than fat restriction (Volek et al. 2009).

1.17.2 Exercise and weight loss

Establishing progressive weight loss requires a consistent state of negative energy balance, therefore increasing energy expenditure through exercise should also promote weight loss.

However, a recent meta-analysis suggests that exercise alone is less effective than diet, or combined diet and exercise interventions (Franz et al. 2007). Maintenance of reduced body mass requires a state of energy balance and it is suggested that this is most effectively achieved when dietary modification is combined with increased physical activity (Donnelly et al. 2009). The potential metabolic benefits of exercise have already been outlined in this chapter and a recent Cochrane review has supported the positive impact of exercise on 'metabolic fitness' independent of reduced body mass (Macfarlane & Thomas 2009; Shaw et al. 2006). Therefore, Chapter 5 of this thesis aims to address the metabolic impact of a seven-week exercise programme and in particular will examine the mechanisms of improved 'metabolic health' with respect to the energy deficit of the intervention and independent of this factor. In addition, Chapter 6 of this thesis will examine the differences between predicted and observed reductions in fat mass in response to an exercise intervention with particular focus on the role of behavioural compensation for the energy deficit of exercise and associated metabolic processes which may contribute to an exercise-mediated reduction in body fat.

1.18 Clinical approaches to treatment of insulin resistance

Despite a growing appreciation of the multiple metabolic defects inherent to the development of insulin resistance, clinicians have had relatively few pharmacological options available to treat those with a pre-diabetic insulin resistant state. Acarbose, an alpha-glucosidase inhibitor has been shown to reduce post-prandial hyperglycaemia and its use in patients with impaired glucose tolerance appears to reduce progression to T2D (Chiasson et al. 2002). Concerns have however been expressed about the validity of the methodology and data analysis in this trial (Sawicki & Kaiser 2004) and in addition, acarbose is generally poorly tolerated by patients due to gastrointestinal side effects (Catalan, Couture, & LeLorier 2001).

Metformin has also been shown to reduce progression to T2D from IGT in the Diabetes Prevention Program (Knowler et al. 2002) and the Diabetes Prevention Study (Tuomilehto et al. 2001). Metformin is considered to have pleotropic effects on insulin resistance including reduction of dietary carbohydrate absorption, reduced hepatic glucose output and increased skeletal muscle insulin sensitivity (Setter et al. 2003). The thiazolidinedione class of drugs are also recognised to have insulin-sensitising effects and have been shown to prevent progression to diabetes (Gerstein et al. 2006), but concerns exist about long-term therapy with respect to possible cardiovascular (Nissen & Wolski 2007) and bone toxicity (Kahn et al. 2006). These concerns may be particularly important in the context of the potential duration of therapy, and with limited knowledge about the persistence of the preventative effect after cessation of drug therapy (Crandall et al. 2008).

Lifestyle intervention trials have frequently combined dietary advice with measures to increase physical activity and in those studies where groups received specific diet or exercise advice both were still efficacious, but not as effective as when combined (Knowler et al. 2002; Pan et al. 1997; Tuomilehto et al. 2001; Ramachandran et al. 2006). These interventions are often associated with weight loss, hence the greater success of a combined diet and exercise approach. However not all exercise interventions produce weight loss, but frequently continue to be associated with improved metabolic health (Ross et al. 2000; Simmons et al. 2008). Indeed, a recent study by Burton and colleagues (2008) highlighted the metabolic benefit of exercise, showing that exercise with replacement of the energy deficit incurred was still beneficial, reducing post-prandial insulinaemia and increasing fat oxidation (Burton et al. 2008).

Obesity surgery is proven to promote large reductions in body mass and is also associated with improved metabolic fitness. Sjöström and co-workers have more than ten years of follow up data for Swedish subjects who had bariatric surgery and have reported sustained

reductions in mortality and morbidity from diabetes and cardiovascular risk (Sjostrom et al. 2004). Despite the initial high economic cost of bariatric surgery it is a cost-effective way of treating severe obesity and may be an appropriate modality in the treatment of the non-obese patient with T2D who is judged to be ‘metabolically unhealthy’ and whose glycaemic control has been poor despite aggressive management with conventional agents (Picot et al. 2009).

1.19 Summary

In conclusion, the literature review detailed in this chapter has explored the increasing prevalence of obesity and insulin resistance, with the subsequent risk of T2D and cardiovascular disease, as well as the aetiology of insulin resistance and possible methods of preventing its progression to chronic disease. The aetiology of insulin resistance in many individuals is likely to be a consequence of genetic predisposition such as a family history of diabetes or an ethnic group with characteristic tendencies and exposure to an environment which promotes a sedentary lifestyle with a ‘Westernised’ diet. In some cases, the risk of diabetes may be attributable to early origin factors although these too may be influenced by parental history and subsequent environmental conditions.

The pathophysiology of insulin resistance is complex and multifactorial but consistent observations have suggested that adipose tissue factors such as NEFA and adipokines have an important role in determining insulin resistance in liver, muscle, adipose tissue and the vascular endothelium. Insulin resistance results in an inability to utilise energy substrates efficiently, leading to a chronic, progressive deterioration in metabolic function. The development of insulin resistance may have relatively poorer health outcomes in women compared to men and given the proven efficacy of lifestyle interventions in insulin resistant states, women may represent an important group to target for intervention. In addition, those with a family history of T2D are a clearly defined population who are at greater risk of

chronic disease and may display early metabolic defects. These defects may be acutely sensitive to exercise intervention and therefore targeting high risk subjects, such as those with a parental history of T2D may represent a cost-effective strategy to prevent this disease.

Exercise is of benefit in the treatment of insulin resistance and overweight and obesity, however the mechanisms by which these benefits occur are incompletely understood, particularly with respect to benefits independent of energy deficit. In addition, exercise may impact upon metabolic health through different mechanisms depending upon the physical and genetic characteristics of the individual as well as alterations in other aspects of lifestyle in response to a change in physical activity. With respect to exercise for the promotion of weight loss, it is unclear whether ‘failure’ to achieve predicted weight loss with a known energy deficit is a consequence of behavioural compensation or a consequence of acquired or innate factors.

The hypotheses of the studies contained within this thesis are as follows. Firstly, that sedentary, pre-menopausal women with a parental history of T2D are more insulin resistant than matched controls and that despite normoglycaemia, other metabolic defects are present which are involved in the aetiology of insulin resistance. Secondly, that a familial history of diabetes is evidence of a ‘thrifty genotype’ which is sensitive to increases in physical activity and that exercise will have a greater impact upon those women with a family history of T2D. Thirdly, that any potential augmented response to exercise in women with a family history of T2D is associated with either enhanced metabolic or physical responses to exercise or that in these subjects exercise influences separate metabolic pathways. Finally, that individual variation in exercise-mediated reduction in fat mass is a consequence of behavioural compensation, differences in innate metabolic responses to exercise or a combination of both.

Therefore the aims of this thesis are as follows: In Chapter 3: to determine the physical and metabolic characteristics of sedentary, normoglycaemic pre-menopausal women with a parental history of T2D compared to matched subjects without a familial history of T2D. In addition, to determine the influence of lifestyle and adiposity as potential mediators of metabolic dysfunction in those with evidence of insulin resistance and whether mediators of insulin resistance are dependent on a family history of T2D. In Chapter 4: to determine the metabolic impact of a seven-week aerobic exercise intervention on the same two cohorts and to determine whether accompanying changes in physical, metabolic or lifestyle parameters provide evidence as to the pathways by which exercise might improve metabolic health. In Chapter 5: to examine the contributors to an exercise-mediated increase in metabolic fitness, whether there are different mechanisms of action depending upon a familial predisposition towards T2D and to what extent improved metabolic health is achieved independently of reduced fat mass and energy deficit. In Chapter 6: to explore the individual variation in exercise-mediated reduction in fat mass and particularly to examine both the role of possible behavioural compensation and potential innate exercise-responsive pathways.

CHAPTER 2

GENERAL METHODS

2.1 Introduction

This chapter describes the methods employed in recruiting volunteers for the study and the measurement of the anthropometric, metabolic and cardio-respiratory variables required in the study protocol. This chapter also discusses the exercise intervention and the procedures involved in monitoring adherence to the exercise regime. This study was conducted with the ethical approval of the Ethics Committee of North Glasgow Hospitals University NHS Trust and was registered with the National Institute for Health (NIH) in the United States of America (Reference No. NCT00268541). Registration details can be viewed at the following website; <http://www.clinicaltrials.gov/ct>.

2.1.1 Inclusion & exclusion criteria

Volunteers were deemed suitable to participate in the trial having met the following criteria; Female, aged between 20 and 45 years old with regular menstrual cycle, non-smoking, systolic blood pressure <160 mmHg, diastolic blood pressure <90mmHg, sedentary lifestyle (less than 1 hour planned physical activity per week, and sedentary job), in good general health without diabetes or a past history of cardiovascular disease. Volunteers were not excluded from the study if they were on prescribed medication, provided that their prescription was stable and did not include medications which were recognised to modulate glucose or lipid homeostasis. Volunteers were allocated into one of two study groups according to family history of type 2 diabetes. Volunteers with a parent who had developed type 2 diabetes prior to the age of 65 years were designated ‘Offspring’, and those with no first or second degree relative with diabetes prior to the age of 65 years were designated as ‘Controls’.

2.1.2 Recruitment, Screening & Selection

Volunteers were recruited from the Greater Glasgow area (See Section 2.14.1, Power Calculation). A variety of media were utilised to publicise the study. Initial recruitment was generated from an advertisement in the Glasgow University Newsletter, further exposure occurred through an article in the Evening Times newspaper which coincided with a press release from the British Heart Foundation. Recruitment posters were displayed around the Glasgow University main campus, and associated campuses across Glasgow (e.g. Jordanhill and Gartnavel campus). A number of general practitioners who provide primary care for patients with type 2 diabetes through the Glasgow Diabetes Service agreed to display recruitment posters in their surgery waiting rooms. Posters aimed specifically at recruiting subjects with a family history of type 2 diabetes were displayed in outpatient clinic areas and diabetic day units of Stobhill Hospital, Gartnavel General Hospital and Glasgow Royal Infirmary. Potential volunteers could access information about the study through a weblink sited on the University Sports and Recreation website. In addition to this, volunteers were able to provide basic contact details and note their interest by submission of a webform which was emailed to the principal investigators. A small number of volunteers was also recruited through an internet football discussion forum. Information on the study was disseminated by word-of-mouth, by both the investigators and volunteers themselves, and was responsible for a small proportion of the total number of subjects recruited.

Interested volunteers were initially contacted by telephone to provide information and to arrange a formal screening visit to the Metabolic Investigation Suite in the Institute of Diet, Exercise and Lifestyle (IDEAL) in the West Medical Building of the University of Glasgow. Volunteers also completed a health questionnaire (Appendix A1) to exclude significant health problems and to determine any important family history. They were provided with an information sheet (Appendix A2), given an oral presentation which explained the study and were then encouraged to give feedback and ask questions. Informed consent was obtained in triplicate. One copy was given to the volunteer for reference, one copy was retained as part of

an individualised case record for the volunteer (Appendix A3), the third copy was sent to the volunteer's general practitioner (GP) as part of an information pack. The information pack contained an explanatory letter (Appendix A4), with a copy of the consent form (Appendix A3) and the information sheet (Appendix A2).

Volunteers attended after a 12-hour overnight fast. Blood samples were obtained by routine venepuncture using the Vacutainer™ (BD Vacutainer Systems Preanalytical Solutions, Plymouth, PL6 7BP) system. Fasting plasma glucose (hexokinase method), lipids - total cholesterol (cholesterol oxidase method), triglycerides (glycerol oxidase method), HDL cholesterol (detergent/cholesterol oxidase method) and LDL cholesterol (Friedewald equation (Friedewald, Levy, & Fredrickson 1972), hepatic enzymes (alanine aminotransferase, aspartate aminotransferase (international federation of clinical chemistry method), bilirubin (diazotization spectrophotometry), alkaline phosphatase (AMP colorimetry), albumin (BCP quantitative colorimetry), and gamma-glutamyl transferase (carboxy nitroanilide spectrophotometry), renal - sodium, potassium, chloride (all by ion selective electrode method) urea (urease method) and creatinine (Kinetic Jaffe spectrophotometry), and thyroid (thyroid stimulating hormone, free T4 both by immuno-enzymatic reaction) function were analysed using Abbott commercial reagents (Abbott Laboratories, Illinois, USA) on C8000 or Architect analysers (Abbott Laboratories, Illinois, USA) in the NHS biochemistry laboratories at Glasgow Royal Infirmary.

A basic physical examination was performed, including cardiac auscultation to exclude significant murmurs, and auscultation of carotid arteries to exclude carotid bruits. Two volunteers were noted to have previously undiagnosed cardiac murmurs. These two volunteers underwent echocardiography at the Cardiology Department of the Western Infirmary, Glasgow and in both cases no significant valvular abnormality was detected. One volunteer was noted to have a right carotid bruit and had Carotid Doppler Ultrasonography

performed in the Acute Stroke Unit at the Western Infirmary, Glasgow, which also excluded abnormal carotid flow. It was necessary to perform carotid imaging as the Complior SP system uses a probe which applies pressure over the carotid vessels and could theoretically precipitate a cerebral thromboembolic event arising from a vessel containing an atherosclerotic plaque.

All subjects performed a Bruce Protocol Exercise Tolerance Test (BPETT) (Bruce, Kusumi, & Hosmer 1973). This test is an incremental exercise test consisting of 3-minute stages which produce a stepwise increase in workload by increasing both treadmill speed and gradient (Figure 2.1). The test was performed with electrocardiograph and blood pressure monitoring, and was performed and interpreted by the author (a registered medical practitioner with Advanced Life Support (ALS) accreditation). All volunteers achieved an adequate workload on BPETT and remained free of symptoms and electrocardiographical signs of coronary artery insufficiency.

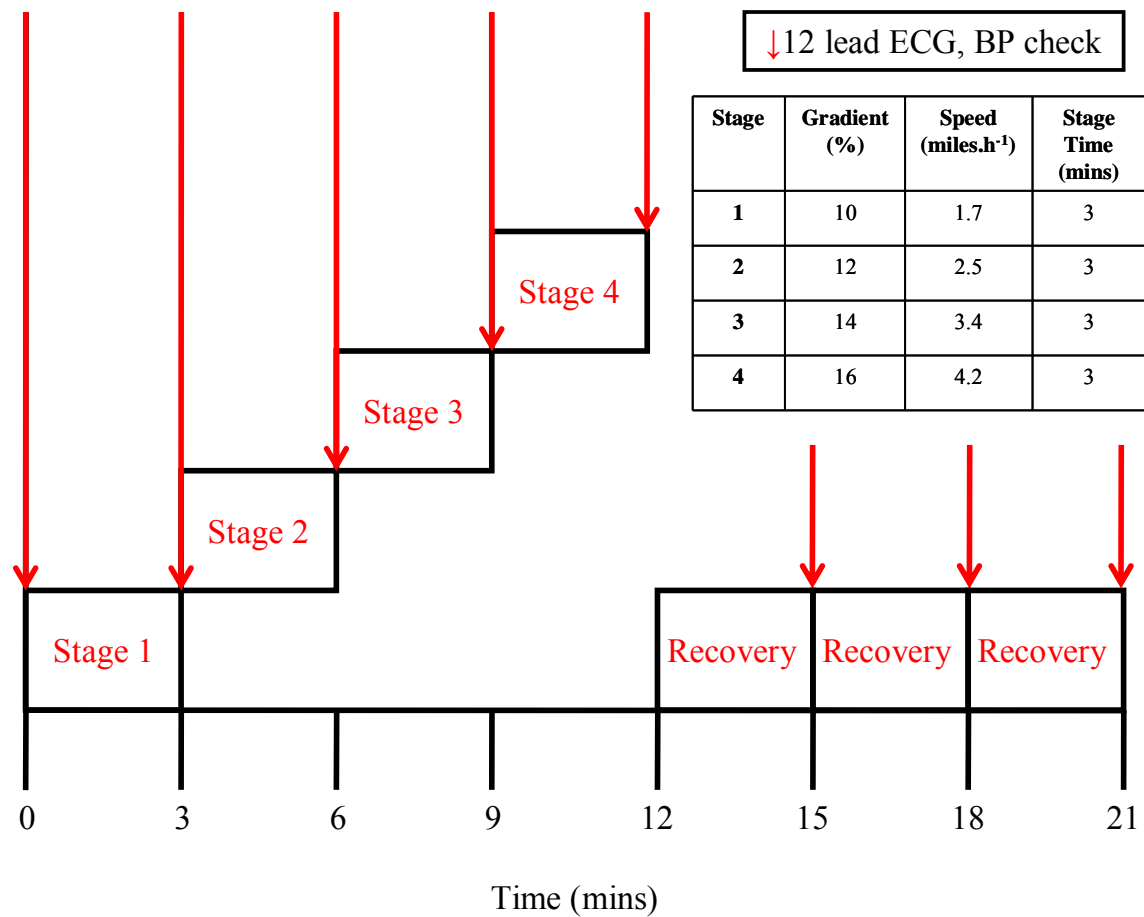


Figure 2.1 Diagrammatic representation of Bruce Protocol Exercise Tolerance Test. Table insert displays changes in gradient and speed of treadmill at given stage of protocol.

Figure 2.2 illustrates the numbers of people involved in the study, including dropouts and exclusions.

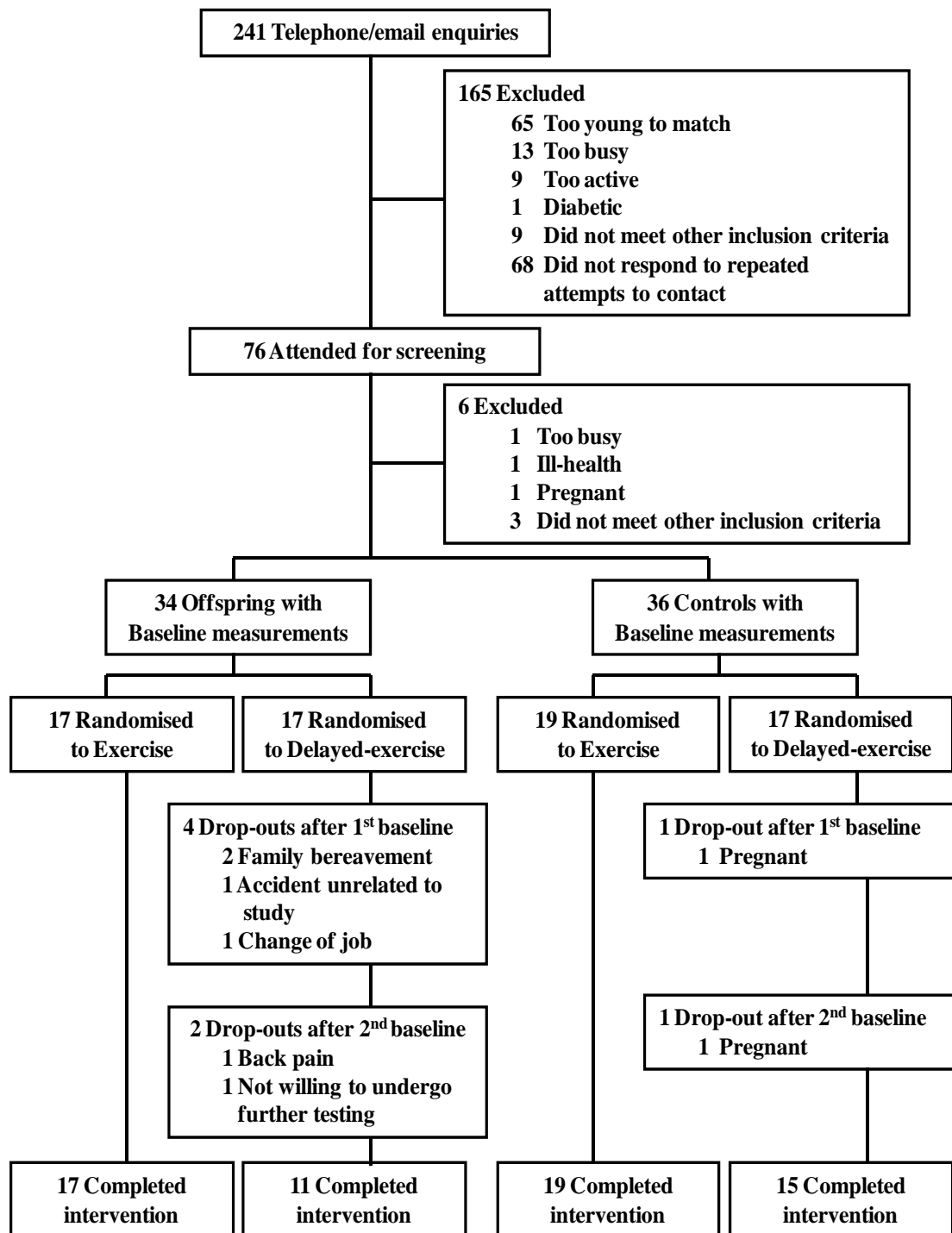


Figure 2.2 Flow chart of volunteer recruitment and randomisation with details of dropouts and exclusions at each stage of the study.

2.1.3 Study Design

The two groups (Offspring and Controls) were screened and investigated in parallel and participants were randomly allocated into one of two subgroups. The 'Delayed Exercise' subgroup was designed to provide a study period of 'usual activity', with metabolic, anthropometric and cardiorespiratory testing before and after a 7-week period where subjects were encouraged to maintain their usual lifestyle (Figure 2.3). This provided a control period to determine whether a 'study effect' influenced any of the studied variables. Subjects in the Delayed Exercise group entered a 7-week exercise programme after completing their 7-week period of 'usual activity', and had one further testing period after this. Figure 2.3 displays the timeline of testing and intervention in the Delayed Exercise group.

The 'Exercise' group was tested in the week prior to commencing the 7-week exercise programme. Repeat exercise testing was performed in week 7 of the intervention, with two post-intervention assessments of metabolic parameters in week 8. The first post-intervention metabolic assessment occurred within 15-24 hours of completion of the final exercise session of the intervention. After an interval of 3 days, during which time volunteers were asked to avoid planned exercise, a further metabolic assessment was performed (Figure 2.4) to ascertain the chronic versus the acute effects of exercise training on the measured variables. The volunteers were randomised to one of the two intervention arms using the 'randbetween' function in an Excel spreadsheet (Microsoft Corp. Redmond, WA, USA). Volunteers whose entry into the spreadsheet generated an odd number between 1 and 10 were placed in the Delayed Exercise group and those who generated an even number between 1 and 10 were placed in the Exercise group.

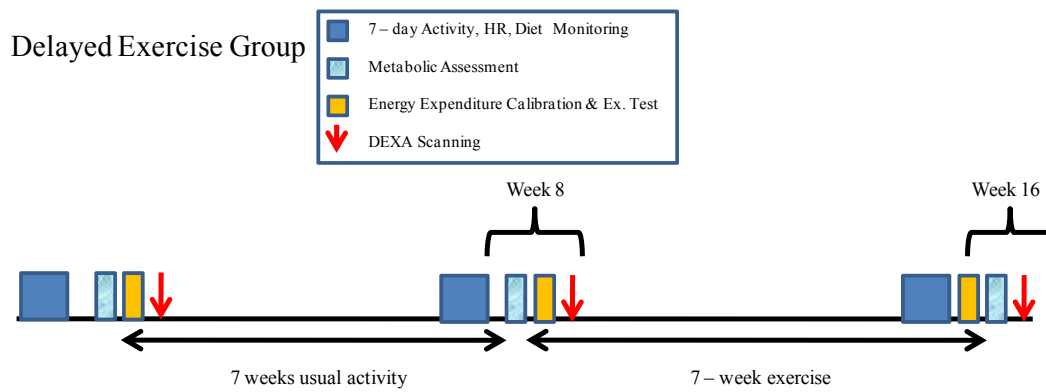


Figure 2.3 Study design for Delayed Exercise group.

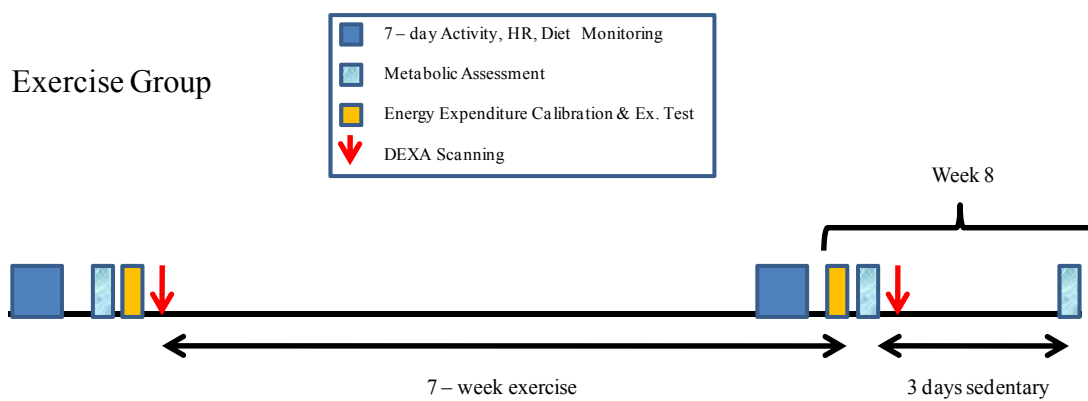


Figure 2.4 Study design for Exercise group.

Volunteers were asked to attend for a number of separate appointments at baseline, and subsequent re-testing intervals. As exercise acutely influences a number of metabolic variables, such as insulin sensitivity and lipid metabolism (Koopman et al. 2005; Magkos et al. 2007; Magkos et al. 2008; Wooten, Biggerstaff, & Anderson 2008), it was important to structure the timing of the submaximal fitness tests at baseline in the Exercise group, and at both baseline and ‘after usual lifestyle’ period in the Delayed Exercise group, to prevent an impact on the metabolic testing.

2.2 Anthropometry and Body Composition

2.2.1 Height

Height was measured using a stadiometer (Invicta Plastics Ltd, Leicester, UK). The volunteer was measured barefoot, with their back positioned against a fixed backboard and their arms relaxed in the lateral position. The head was also positioned against the backboard, with the line of eyesight perpendicular to the backboard. Measurement was performed when the volunteer was positioned and relaxed, and a moveable headboard was lowered on to the top of the head with light pressure allowing hair compression. The investigator applied gentle upwards pressure underneath the angle of the mandible and measurement was made to the nearest 0.01 metre.

2.2.2 Body Mass

Body mass was measured using a manual balance weighing scale (Avery, Birmingham, UK). The same scale was used for all volunteers throughout the study. Subjects were weighed wearing lightweight clothing (e.g. shorts and t-shirt) without wearing shoes. Extraneous jewellery and clothing was removed prior to weighing. Body mass was measured with both feet flat on the balance and with arms positioned in the lateral position. Measurement was made to 0.05 kg.

2.2.3 Skinfold Thickness

All measurements of skinfold thickness were performed in private, by the same investigator on each occasion. Measurements were made to 0.1 mm using Harpenden skinfold callipers (Cranlea & Company, Birmingham, UK). The same callipers were used throughout the study. The skinfold was held between the investigator's thumb and index finger, the callipers were applied and the measurement made after pressure had been applied for between 5 and 8

seconds. The left hand side of the body was used for measurement, and measurements were made with the volunteer standing up. Biceps skinfold was marked with the elbow flexed at an angle of 90°, at the midpoint between the most lateral point of the acromion and the inferior border of the olecranon. Once marked, the arm was allowed to hang loosely with the palm facing anteriorly, and the measurement made in the midline of the biceps muscle, at the level of the mark. Triceps skinfold was measured at the same level as the biceps measurement, on the posterior aspect of the arm, in the midline. The subscapular skinfold was measured along the line of natural skin cleavage inferior to the scapula, with both arms hanging loosely by the volunteer's side. The suprailiac fold was measured above the iliac crest, on a diagonal fold beginning at the anterior axillary line.

Skinfold measurements were used to illustrate regional adiposity and were not used to determine body density, or body fat. Coefficients of variation were 1.9% for biceps, 0.8% for triceps, and 0.7% for both subscapular and suprailiac skinfolds.

2.2.4 Waist & Hip Circumference

Circumferences were measured using a non-elastic tape measure, and measurement was made with the abdominal muscles relaxed, at the end of normal expiration. Both hip and waist measurements were taken three times, and an average of the three readings was noted. Waist circumference was measured horizontally around the waist, at a point midway between the superior border of the ilium and the inferior border of the lateral margin of the ribs (costal margin). Hip circumference was measured around the point of maximal width around the hip region, at approximately the level of the pubic symphysis. The same investigator performed the measurements on each volunteer, on every occasion. Waist to hip ratio was determined by dividing the waist circumference by the hip circumference.

2.2.5 Dual X-Ray Absorptiometry (DEXA) Scanning

Dual X-Ray Absorptiometry (DEXA) scanning is recognised as an accurate method of measuring body composition (Gallagher & Song 2003). DEXA uses X-ray beam radiation of two energies, and is able to interpret the differential attenuation of the two energies to identify bone, lean and fat tissue. Scans were performed by an experienced radiographer (Senior II Grade) using a GE Medical LUNAR Prodigy DEXA scanner (GE Healthcare Diagnostic Imaging, Slough, Berkshire, UK), in the Radiology Department of the Royal Hospital for Sick Children, Yorkhill, Glasgow. Scan data was interpreted using the generic software.

DEXA scanning was carried out at intervals as described in Figures 2.3 – 2.4. The amount of radiation used in a DEXA scan is approximately 0.0007 mSv, equivalent to 10% of one day of background radiation. For further information, volunteers were advised that the amount of radiation delivered during one DEXA scan was fifty times less than one chest x-ray.

2.3 Metabolic Testing

2.3.1. Protocol of metabolic testing

Volunteers attended the Metabolic Investigation Suite after a 12-hour overnight fast. Those volunteers randomised to the Exercise group who were attending for the first metabolic testing after the exercise intervention were asked to complete their final exercise session 15-24 hours prior to testing. Following a 10-minute rest lying on a couch, a 20-minute continuous expired air collection was performed using a ventilated hood system (Deltatrac Metabolic Monitor, Datex Engstrom, Kent, UK). Volunteers remained in a supine position in a quiet room after the completion of the expired air sampling and had blood pressure measurement using an automated blood pressure monitor (Omron Healthcare UK Limited, Milton Keynes, UK) (section 2.3.5). Whilst still recumbent, peripheral pulse wave velocity

was assessed using the Complior SP system (Artech Medical, Pantin, France). A cannula was introduced into an antecubital vein, and after a 10-minute interval, a fasting blood sample was taken. In order to gain adipose tissue for analysis of gene expression (data not presented in this thesis), volunteers had a 0.5-1 gram sample of subcutaneous abdominal adipose tissue obtained by liposuction technique from an area lateral to the umbilicus. Since the data derived from these biopsies is not presented in this thesis, the method relating to the biopsy procedure is not described in detail, but included to explain the delay between fasting blood sampling and administration of the oral glucose load. After completion of the liposuction biopsy, the volunteers underwent an oral glucose tolerance test (OGTT). A second period of 20-minute continuous expired air collection was made using the ventilated hood following the 120-minute blood sample, and a further assessment of blood pressure and peripheral pulse wave velocity was made (Figure 2.5). Since volunteers had engaged in a prolonged fast, they were offered lunch after concluding the metabolic tests.

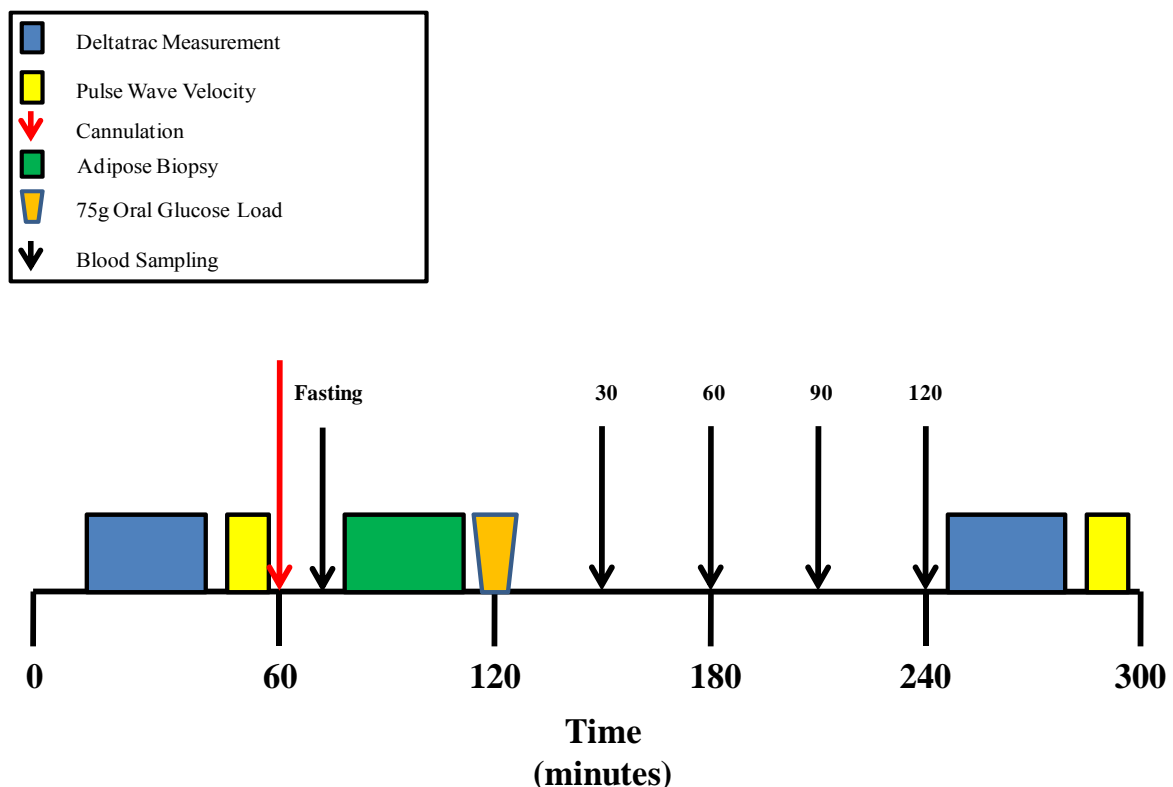


Figure 2.5 Timeline illustrating metabolic testing protocol.

2.3.2 Metabolic Rate & Energy Substrate Utilisation

Resting metabolic rate (RMR) was measured in the morning, after a 12-hour fast and 24-hour abstention from exercise, using the Deltatrac Metabolic Monitor (Datex Engstrom, Kent, UK). The Deltatrac was switched on 30 minutes prior to use, and calibrated for ambient atmospheric pressure using a traditional barometer. To ensure accurate assessment of FEO_2 and FECO_2 the gas analyser component of the Deltatrac was calibrated against a known standard gas (95% O_2 , 5% CO_2) (Quick Cal™ Calibration Gas, Datex-Ohmeda, Cranlea & Company, Birmingham, UK). Environmental conditions were controlled to ensure a quiet room, with minimal external stimuli and a temperature of 24°C. Volunteers reclined on an examination couch, with the hood in position. No measurements were made for a ten-minute period to allow the volunteer to relax and to acclimatise to both the hood, and the temperature of the room.

Measurements were recorded every 60 seconds for 20 minutes. This system uses a ventilated hood which is placed over the subject's head and neck, with an attached plastic skirt which covers the upper torso. The ventilated hood has a cranial aperture which allows the incursion of ambient air, and a caudal aperture which is attached by tubing to the main body of the Deltatrac (Figure 2.6). The instrument draws air through the hood at a constant flow rate and from this and the fraction of expired CO_2 (FECO_2) and O_2 (FEO_2) is able to calculate both rate of oxygen consumption ($\dot{\text{V}}\text{O}_2$) and rate of carbon dioxide production ($\dot{\text{V}}\text{CO}_2$). One minute average values of $\dot{\text{V}}\text{O}_2$ and $\dot{\text{V}}\text{CO}_2$ were recorded and an average of the final 15 minutes of measurement was used to calculate respiratory exchange ratio (RER), and rate of energy substrate utilisation and energy expenditure, using indirect calorimetry equations derived by Frayn (Frayn 1983). (see section 2.3.3). Measurement of metabolic rate and substrate utilisation was performed in the same manner, 2 hours following glucose ingestion in the oral glucose tolerance test. Continuous measurement of expired air during the whole period of the OGTT may have allowed more thorough analysis of diet-induced thermogenesis, however the ventilated hood is uncomfortable for prolonged periods. In

addition, the majority of the volunteers were in full or part-time employment and were keen to use the 2-hour period to perform sedentary work-related tasks (e.g. checking emails). In retrospect, a further 20-minute expired air collection at 1 hour post-glucose ingestion might have provided evidence of subtle differences in diet-induced thermogenesis and metabolic inflexibility.



Figure 2.6 Photograph of Deltatrac Metabolic Monitor with male volunteer.

2.3.3 Indirect calorimetry calculations

Mean values for $\dot{V}O_2$ and $\dot{V}CO_2$ over the 20-minute measurement period were used in the indirect calorimetry equations derived by Frayn (Frayn 1983). Frayn's equations require a value for nitrogen excretion to provide an estimation of protein oxidation. Urinary nitrogen excretion was not measured during this study, therefore a constant rate of nitrogen excretion of $0.00011 \text{ g.kg}^{-1}.\text{min}^{-1}$ was assumed in accord with published values in the literature (Flatt et al. 1985; Melanson et al. 2005).

$$N(\text{g.min}^{-1}) = 0.00011 \times \text{body mass (Equation 2.1)}$$

Frayn's equations allow calculation of $\dot{V}O_2$ and $\dot{V}CO_2$ according to equations 2.2 and 2.3 respectively.

$$\dot{V}O_2 \text{ (l.min}^{-1}\text{)} = 0.746 \text{ CHO} + 2.03 \text{ F} + 6.04 \text{ N (Equation 2.2)}$$

$$\dot{V}CO_2 \text{ (l.min}^{-1}\text{)} = 0.746 \text{ CHO} + 1.43 \text{ F} + 4.89 \text{ N (Equation 2.3)}$$

In these equations, CHO is carbohydrate (grams), N is the constant for nitrogen excretion (grams) and F is fat (grams). Combining these equations with a correction for protein oxidation allowed calculation of non-protein oxygen consumption ($NP\dot{V}O_2$) and non-protein carbon dioxide production ($NP\dot{V}CO_2$), and therefore allowed the non-protein respiratory exchange ratio (NPRER) to be determined. Therefore, equations 2.4 and 2.5 determine $NP\dot{V}O_2$ and $NP\dot{V}CO_2$ respectively, whilst equation 2.6 determines NPRER.

$$NP\dot{V}O_2 \text{ (l.min}^{-1}\text{)} = \dot{V}O_2 - 6.04 \text{ N (Equation 2.4)}$$

$$NP\dot{V}CO_2 \text{ (l.min}^{-1}\text{)} = \dot{V}CO_2 - 4.89 \text{ N (Equation 2.5)}$$

$$NPRER = NP\dot{V}CO_2 / NP\dot{V}O_2 \text{ (Equation 2.6)}$$

Use of protein corrected values allowed calculation of individual energy substrate oxidation (Equations 2.7 – 2.9).

$$\text{Fat Oxidation (g.min}^{-1}\text{)} = (N\dot{V}O_2 - NP\dot{V}CO_2) / 0.6 \text{ (Equation 2.7)}$$

$$\text{Carbohydrate Oxidation (g.min}^{-1}\text{)} = (\text{NP}\dot{\text{V}}\text{O}_2 - 2.03 \times \text{F Ox.}) / 0.746 \text{ (Equation 2.8)}$$

$$\text{Estimated Protein Oxidation (g.min}^{-1}\text{)} = \text{N} \times 6.25 \text{ (Equation 2.9)}$$

Total energy expenditure was the sum of energy obtained from fat, CHO and protein oxidation. Energy obtained from individual substrate oxidation was determined by the product of the energy density of each substrate and the amount of substrate oxidised (Equation 2.10).

$$\text{EE (kJ)} = (\text{fat} \times 39.0) + (\text{carbohydrate} \times 15.5) + (\text{protein} \times 17.0) \text{ (Equation 2.10)}$$

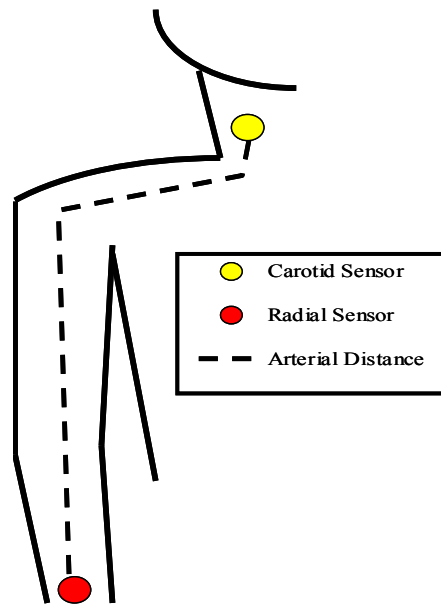
2.3.4 Blood Pressure

The controlled environment and supine positioning described in section 2.3.2, conforms to the European Society of Hypertension guidelines on blood pressure measurement (O'Brien et al. 2003). An automated monitor was used (Omron Healthcare UK Limited, Milton Keynes, UK), and the lowest value of three readings was taken as the recorded value.

2.3.5 Pulse Wave Velocity

The Complior SP system (Artech Medical, Pantin, France) was used to assess peripheral pulse wave velocity (PWV). Assessment of peripheral PWV by carotid –radial measurement was a less intimate examination for female volunteers than central PWV by carotid – femoral measurement. The main investigator was male, therefore central measurement would have required a chaperone. Anthropometric data (height, body mass, waist and hip circumference), and blood pressure measurements were entered into the Complior SP generic software prior to each measurement of PWV. The carotid and radial arteries were identified by manual

palpation, and a sensor applied over each. The manufacturers supply clamps which hold the sensors in place with light pressure. The radial clamp was used to allow the operator a free hand, however the carotid clamp was found to be uncomfortable for the volunteer and easily dislodged leading to loss of sensor signal, therefore the carotid sensor was held in place by the operator's left hand. Both sensor positions were manipulated until a clear waveform was identified on the computer screen. After maintenance of good quality signals from both areas for approximately 10 seconds, a foot pedal was depressed which triggered measurement of the time delay of a single pulse wave being detected by the carotid sensor and the radial sensor. The time delay measured by the Complior SP device is the time taken for a single waveform generated by one left ventricular systole to travel from one site in the arterial tree to another more distal site (in this case between the carotid and radial arteries). The distance between the two sites was measured by a non-elastic tape measure, and this distance was also entered into the Complior SP software (Figure 2.7). This allowed calculation of PWV by the following equation (Equation 2.11).



$$\text{PWV (m.s}^{-1}\text{)} = \text{distance (m)} / \text{sensor – sensor time (s)} \text{ (Equation 2.11)}$$

Figure 2.7 Illustration of Pulse Wave Velocity arterial sensors and measurement of arterial distance.

Six PWV measurements were made, and the average value recorded. Measurement of PWV was performed in the fasted state and upon completion of the oral glucose tolerance test (Figure 2.5). Pulse wave velocity is a marker of arterial stiffness (Tomiyama & Yamashina 2010) and correlates with flow-mediated arterial dilatation (FMD) suggesting that it is a surrogate for endothelial function (Jadhav & Kadam 2005). Nitric oxide is derived from vascular endothelium and is a key mediator of vasodilatation throughout the vascular tree, FMD is considered to be a non-invasive marker of local NO bioavailability. Pulse wave velocity is therefore also considered to be indicative of endothelial function and has been shown to be impaired in offspring of patients with T2D (McEleavy et al. 2004). However whilst PWV correlates with FMD, it is a direct measurement of large vessel elasticity and is not a direct measurement of microvascular endothelial function (Tomiyama & Yamashina 2010). Skeletal muscle microvascular function can be measured more directly using invasive techniques such as laser doppler flowmetry, microdialysis or contrast enhanced ultrasound (Clark 2008;Coggins et al. 2001). However, these techniques are considerably more invasive, technically demanding and time-consuming than PWV and have tended to be used in conjunction with insulin infusions which would have been unsuitable for the study protocol. Venous occlusion plethysmography offers a non-invasive and simple technique which has been purported to measure microvascular perfusion (Alexandraki et al. 2006;Woodman et al. 2005). However whilst measurements of reactive hyperaemia in insulin resistance are consistent with measurements made by FMD and are therefore representative of endothelial function, they are actually measurements of total forearm bloodflow and are not isolated measurements of capillary recruitment (Alexandraki et al. 2006;Clark 2008). Unlike PWV, venous occlusion plethysmography does provide an index of vascular function which includes capillary recruitment therefore despite its limitations it may have been a more sensitive method of assessing differences in vascular function in these groups.

2.3.6 Fasting Blood Sampling

After PWV measurements an 18G cannula was placed in an antecubital vein. After a 10-minute interval, a fasting blood sample was taken using two 10 ml ethylenediamine tetra-acetic acid (EDTA) Vacutainer™ tubes (BD Vacutainer Systems, Plymouth, UK). After the samples were obtained, the cannula was flushed to maintain patency, using 5 mls of 0.9% saline solution.

2.3.7 Oral Glucose Tolerance Test

A 75 g oral glucose load was given to the volunteer. This was prepared using 82.5g of Dextrose Monohydrate (Nutrivit Ltd, Corby, UK) in 340 ml of water, with 10 ml of lemon juice (Jif, Colmans of Norwich, Norwich, UK) to give a total volume of 350 ml. Volunteers were instructed to drink the full volume in a 2-minute period, and a stopwatch was started once the volunteer commenced drinking.

At 30, 60, 90, and 120 minutes after ingestion of the glucose drink a 10 ml blood sample was taken through the cannula, into an EDTA tube. Volunteers were supine at all blood collection points. After each sample the cannula was flushed with 5 ml of 0.9% saline.

2.4 Plasma Preparation and Analysis

2.4.1. Plasma preparation and storage

All blood samples were placed on ice prior to refrigerated centrifugation at 4°C. Samples were centrifuged at 3000 rpm for 15 minutes. Plasma was aspirated after centrifugation using a disposable plastic Pasteur pipette. Fasting plasma was dispensed in 0.5 ml aliquots into labelled 2 ml Eppendorf tubes (Alpha Laboratories Ltd, UK), and frozen at -80°C until analysis. Twelve aliquots were stored from fasting plasma, and six aliquots were stored for each subsequent time point during the oral glucose tolerance test.

2.4.2. Enzymatic colorimetric methods

Lipid assays were performed in the Department of Vascular Biochemistry at Glasgow Royal Infirmary. Total cholesterol, HDL-cholesterol and triglycerides were analysed by enzymatic colorimetric methods using commercially available kits (Roche Diagnostics GmbH, Mannheim, Germany). LDL-cholesterol concentrations were determined using the Friedewald equation (Friedewald, Levy, & Fredrickson 1972). NEFA concentrations were determined by enzymatic colorimetric methods, using a commercially available kit (Wako Chemicals GmbH, Neuss, Germany). Glucose was assayed using the hexokinase method, utilising a Randox kit (Randox Laboratories, Crumlin, Co. Antrim, UK). High sensitivity C-reactive protein (hsCRP) was measured using an immunoturbidimetric assay (Randox Laboratories, Crumlin, Co. Antrim, UK). All assays were performed on an ILAB™ 600 clinical chemistry analyzer (Instrumentation Laboratory, USA). Analysis was performed by colleagues at the Department of Vascular Biochemistry. Alanine aminotransferase (ALT), Gamma Glutamyl Transferase (GGT) and 3-hydroxybutyrate (3-OHB) were assayed in the biochemistry laboratory at IDEAL, University of Glasgow using a Cobas Mira Plus (ABX Diagnostics, France). ALT, GGT and 3-OHB were measured using commercial kits (Randox Laboratories, Crumlin, Co. Antrim, UK) by a research assistant in IDEAL. All samples for

each participant were analysed in a single analyser run. The accuracy and precision of the assays was monitored using quality control sera (Roche Diagnostics GmbH, Mannheim, Germany. Randox Laboratories Ltd, Co. Antrim, Ireland. Wako Chemicals GmbH, Germany). Coefficients of variation were < 3.1% for colorimetric assays.

2.4.3. Enzyme-linked immunoassays

All enzyme-linked immunoassay (ELISA) procedures were based on a 'sandwich' technique. Commercially produced plates were used for all ELISA. The wells of the plates were coated with a monoclonal antibody to the protein of interest. The addition of plasma to the wells bound protein to the antibody, with unbound molecules removed by washing. A second antibody specific to another area of the protein was then added, with unbound molecules removed by a second washing. The second antibody was linked to an enzyme which would catalyse the conversion of a nonfluorescent substrate to a fluorescent product. The intensity of the subsequent fluorescence was directly proportional to the amount of protein in the initial sample (Stryer 1988).

Insulin was measured using a commercially available ELISA with <0.01% cross-reactivity with proinsulin (Mercodia, Uppsala, Sweden). Adiponectin, resistin, leptin, IL-6 and TNF α were analysed using commercially available kits (R & D Systems Europe, Abingdon, UK). High sensitivity kits were used for TNF α and IL-6. All samples for each participant were analysed in a single analyser run. The accuracy and precision of the assays was monitored using quality control sera (Mercodia AB, Uppsala, Sweden. R & D Systems Europe, Oxford, UK.). Coefficients of variation were 3.9% for adiponectin, 8.0% for leptin, 7.1% for resistin, 11.3% for IL-6, 9.3% for TNF α , and <4% for insulin.

2.5 Establishment of relationships of heart rate to $\dot{V}O_2$ and $\dot{V}CO_2$ during rest and exercise

Volunteers attended the exercise laboratories at IDEAL as noted in figures 2.3 and 2.4. Volunteers were asked to fast for 2 hours prior to the visit, to prevent elevation of serum lactate as a consequence of intestinal active transport (Frayn 2003). At this visit, volunteers participated in assessment of both cardiorespiratory fitness and measurements of heart rate with expired air collection to establish relationships between $\dot{V}O_2$, $\dot{V}CO_2$ and heart rate during sedentary activities.

2.5.1 Relationship of heart rate to $\dot{V}O_2$ and $\dot{V}CO_2$ during low intensity activities

Volunteers participated in measurements of expired air and heart rate whilst performing low intensity activities. These measurements were employed to provide data on individual relationships (rather than mean values for Offspring and Control groups) between $\dot{V}O_2$ and $\dot{V}CO_2$ which are required for energy expenditure calculations. The importance of an individual calibration technique to determine energy expenditure at low intensity activities is previously recognised (Ceesay et al. 1989; Spurr et al. 1988). Consistent with previous literature (Ceesay et al. 1989; Spurr et al. 1988) three stages were used to assess $\dot{V}O_2$, $\dot{V}CO_2$ and heart rate during low intensity activities. Measurements were made with the volunteer: sitting still, standing still and standing swaying with arms swinging. Hyperventilation may occur at rest when Douglas bags are used for expired air analysis, this may alter $\dot{V}CO_2$ values but does not affect $\dot{V}O_2$ therefore resting data cannot be used to calculate substrate utilisation but calculation of metabolic rate remains robust. Heart rate and expired air analysis was performed as described in section 2.6.1 – 2.6.2.

Volunteers were seated on a stool and were instructed to apply the nose-clips and breathe through the mouthpiece and two-way valve. To allow the volunteer to acclimatise to the environment, mouthpiece and nose-clips, a 5-minute period without expired air collection or heart rate monitoring was performed. A 5-minute expired air collection was then performed. During the last minute of the collection, heart rate was measured. Upon completion of this stage, the volunteer was asked to remove the nose clips and mouthpiece, and offered a drink of water.

Volunteers were then asked to stand up. The mouthpiece and nose-clips were introduced. A one minute run-in period was allowed to clear the mixed expired air and ambient air from the collection apparatus. A further 5-minute expired air collection was performed. Heart rate was recorded in the final minute.

Volunteers were asked to remain standing, transferring weight from one foot to another, with their arms swinging loosely by their side. The mouthpiece and nose-clips were introduced. A further one minute period was allowed to clear the collection tubing of mixed air, before a further 5-minute expired air collection with heart rate recording as described in section 2.6.1 – 2.6.2.

2.6 Assessment of Cardiorespiratory Fitness

Upon completion of the low intensity assessments, volunteers had a 10 minute break at the end of which baseline capillary lactate was measured. The volunteers then performed an incremental submaximal treadmill walking test to assess cardiorespiratory fitness. Capillary lactate sampling was also performed to determine the lactate threshold and to help guide the

duration of the test. Measurements of heart rate and expired air were performed as described in sections 2.6.1 – 2.6.2.

Volunteers had been familiarised with the treadmill during their screening Bruce protocol exercise tolerance test. The same treadmill (Woodway PPS 55Med-I, Woodway GmbH, Weil am Rhein, Germany) was used for all submaximal exercise tests. Each test began with a 5 minute stage with the walking speed set at 3 km.h⁻¹, with a 0% gradient. This stage allowed the volunteer to become familiar with the pattern of mouthpiece and nose-clip application, rate of perceived exertion (RPE) assessment and capillary lactate sample whilst walking on the treadmill.

A stopwatch was started once the volunteer began walking at 3 km.h⁻¹. For the first two minutes of the stage, no expired air was collected. At the start of the third minute, the mouthpiece and nose-clips were introduced, and a one minute expired air collection into Douglas bags was started at the beginning of the 4th minute. A minimum of 15-20 seconds is recommended for expired air collection during exercise at a steady state (Macfarlane 2001). In the 5th minute of the stage, RPE and heart rate were recorded. RPE was recorded using the Borg Scale of 6 to 20, the subject was asked to point at the appropriate point on the scale, and this was confirmed verbally by an investigator (Borg 1973). At 5 minutes, the expired air collection was completed for that stage. The volunteer was assisted in removing nose-clips and mouthpiece and simultaneously, a second investigator took the capillary lactate sample.

Once the lactate sample had been obtained the next stage was commenced, and the treadmill was increased in speed to 5 km.h⁻¹. Real-time capillary lactate analysis was performed during the test to allow detection of the lactate threshold (section 2.6.4). The volunteer continued to walk at 5 km.h⁻¹ for the duration of the test. Subsequent increases in workload were achieved

by a 2% increase in treadmill gradient with each 5 minute stage (Figure 2.8). Concentration of capillary lactate vs. workload was plotted on a graph, and the volunteer was judged to have breached their lactate threshold once an obvious deviation ($> 1 \text{ mmol.l}^{-1}$) had occurred from baseline concentrations.

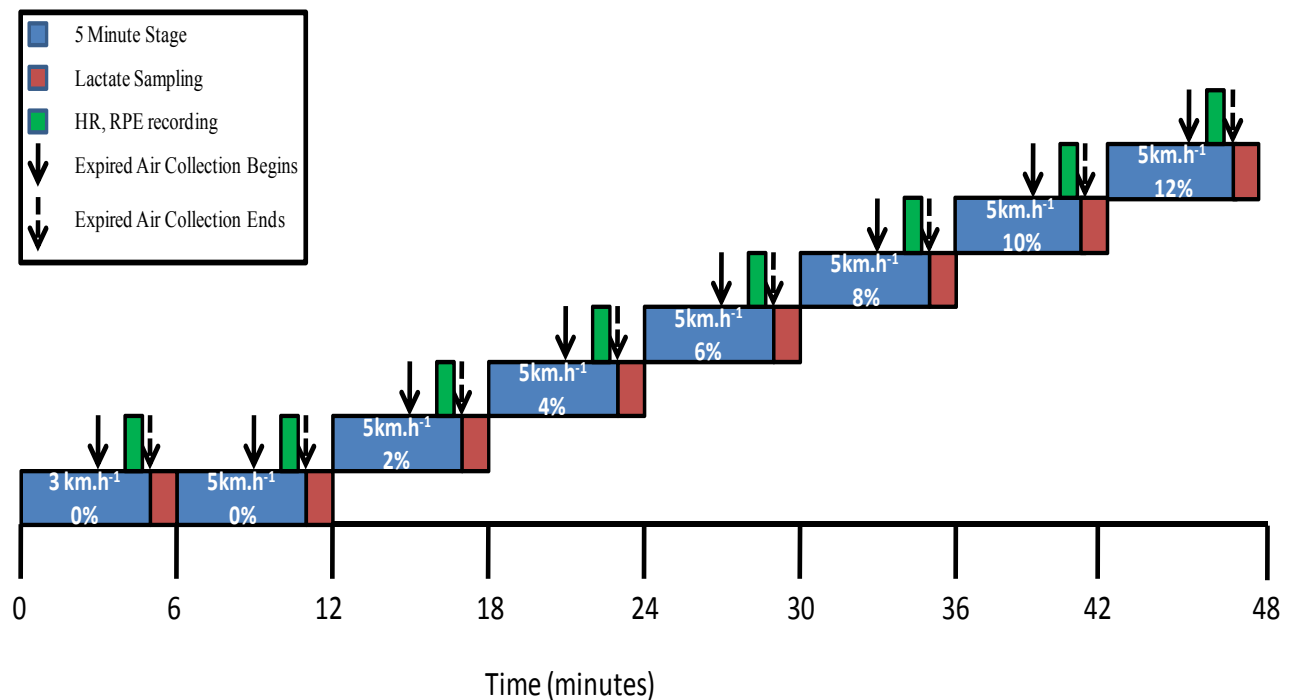


Figure 2.8 Submaximal exercise test protocol.

The submaximal test was terminated once the investigators were convinced that the lactate threshold had been breached, the volunteer asked to stop, or an RPE of greater than 18 was reported.

2.6.1 Measurement of Heart Rate

Heart rate was measured during the establishment of the relationship between $\dot{V}O_2$, $\dot{V}CO_2$ and heart rate at rest, during low intensity activity and the submaximal exercise test using a short-range telemetry system, which utilised a chest wall sensor with transmitter and a watch device which received the signal, and displayed the heart rate (Polar s610i Heart Rate Monitor, Polar Electro Oy, Kempele, Finland). The Polar heart rate monitors were also used

by volunteers during all exercise sessions in the intervention, and for monitoring of heart rate during periods of assessment of habitual physical activity. In the submaximal exercise test, heart rates were recorded every 15 seconds during the 5th minute of each stage of the test. An average of the 4 heart rate recordings was given as the heart rate for that stage. The predicted maximal heart rate was determined by the formula (Fox, III & Haskell 1968):

$$HR_{\max} = 220 - \text{Age} \text{ (equation 2.12)}$$

2.6.2 Expired Air Collection & Analysis

During the low intensity activity measurements and the submaximal exercise test, expired air was collected in Douglas bags, using standard Douglas bag technique (Consolazio CF 1963).

Analysis of expired air was performed in two stages:

Stage 1: 0.5 litres of air was extracted through the sampling port of the Douglas bag at a constant flow rate, controlled by a flow meter. This air was passed into a gas analyser (Servomex 4000 series, Servomex Group Limited, East Sussex, UK) and the percentage fraction of oxygen and carbon dioxide was measured.

Stage 2: The remaining air was extracted by a vacuum at a constant flow rate, through a dry gas meter (Harvard Apparatus Ltd, Kent, UK) which provided a measurement of the volume of expired gas, and through the dry gas meter's thermometer, which provided a measurement of the temperature of the expired air.

Prior to all tests, the gas analyser was calibrated against known reference gases (BOC Gases, BOC Limited, Surrey, UK) and barometric pressure was measured. All gas measurements were corrected to standard room temperature and pressure (STPD) for a dry gas. Values of $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated from expired air using the Haldane transformation of the Fick equation as shown in equations 2.13 – 2.15 (Wilmore & Costill 1973).

$$\dot{V}I = \dot{V}E_{\text{STPD}} \times (100 - \text{Exp. Fraction } O_2 - \text{Exp. Fraction } CO_2) / 79.04 \text{ Equation 2.13}$$

$$\dot{V}O_2 (\text{l} \cdot \text{min}^{-1}) = \dot{V}I \times 0.2093 - (\dot{V}E_{\text{STPD}} \times \text{Exp. Fraction } O_2 / 100) \text{ Equation 2.14}$$

$$\dot{V}CO_2 (\text{l} \cdot \text{min}^{-1}) = (\dot{V}E_{\text{STPD}} \times \text{Exp. Fraction } CO_2 / 100) - \dot{V}I \times 0.0003 \text{ Equation 2.15}$$

2.6.3 Estimation of $\dot{V}O_{2\text{max}}$

Mean heart rate was plotted against calculated $\dot{V}O_2$ for each stage of the submaximal exercise test. In all cases a linear relationship was observed between heart rate and $\dot{V}O_2$ (equation 2.16). The equation of the line describing the relationship between heart rate and $\dot{V}O_2$ was used with the predicted maximal heart rate (HR_{max}) to calculate predicted $\dot{V}O_{2\text{max}}$ (American College of Sports Medicine 1995).

$$HR = m(\dot{V}O_2) + c$$

$$\dot{V}O_{2\text{max}} = (HR_{\text{max}} - c) / m \text{ (equation 2.16)}$$

Measurement of maximal heart rate and $\dot{V}O_{2\text{max}}$ was not performed because this study required continued commitment to the exercise programme and it was felt that an initial maximal test might have dissuaded some subjects from continued participation in the study. In addition, the validity of comparison of pre- and post-intervention maximal testing is more questionable when the group is sedentary at baseline since maximal workload may be falsely perceived (Church et al. 2001; WHO Consultation 1968).

2.6.4 Capillary Lactate Sampling

Capillary lactate was measured in the resting state, prior to the test, and in the last minute of each exercise stage. The initial resting sample was taken after the volunteer's left hand had been immersed for ten minutes in a bath of water heated to 42°C, to ensure increased blood flow and arteriolisation of the sample. The thumb of the left hand was swabbed with an alcohol wipe (Uni-Wipe Alcotip Swabs, Sani System, Enfield, UK). Sampling was performed by digital puncture, using Accu-Chek Softclix lancets (Roche Diagnostics, Welwyn Garden City, UK). The initial blood droplet was wiped away, and a 30-50µL capillary blood sample was drawn into a pre-treated (Heparin/Fluoride/Nitrite) capillary tube (Analox Instruments Ltd, London, UK) by capillary attraction. Lactate was assayed using an Analox GM7 lactate analyser (Analox Instruments Ltd, London, UK).

Capillary lactate samples were taken during the submaximal exercise test. Sampling did not require repeated digital immersion in a water bath, due to increased peripheral blood flow induced by exercise. Samples were taken within one minute of the completion of expired air collection at each stage of the submaximal test. Volunteers were asked to rest their left hand on the edge of the treadmill rail and allow repeat alcohol swabbing, digital puncture and sample collection. Once an adequate sample had been obtained, the volunteer was given a tissue to prevent excessive bleeding.

2.7 Exercise Intervention

2.7.1 Exercise Intensity and Frequency

Volunteers used their heart rate monitors to guide the intensity of their exercise sessions. Volunteers were asked to complete all prescribed sessions exercising within a twenty beat heart rate range which was within 65-80% of their predicted maximum heart rate. This heart rate range is considered to represent a moderate intensity exercise prescription which

promotes increased cardiorespiratory fitness (Pollock et al. 1998). Volunteers were asked to exercise for seven weeks, increasing the number and duration of sessions on a weekly basis. Week 1 of the regime consisted of 3 x 30 minute sessions, and the volunteers completed 5 x 60 minute sessions in weeks 6 and 7 (Table 2.1).

Table 2.1 Duration (minutes) and number of sessions for each week in the exercise intervention.

Week	Duration 1	No. Sessions	Duration 2	No. Sessions
1	30	3	-	-
2	30	2	45	2
3	30	3	45	2
4	45	5	-	-
5	45	3	60	2
6	60	5	-	-
7	60	5	-	-

Both the duration and the heart rate achieved during each session were stored in the memory of the Polar s610i heart rate monitor. Volunteers met a study investigator on a weekly basis, this allowed infrared data transfer from the heart rate monitors to a laptop computer using Polar software (Polar Electro Oy, Kempele, Finland), providing a complete record of all sessions performed in the intervention. In addition, the weekly meeting provided the volunteer with the opportunity to ask questions about the study or exercise regime, identify any problems and arrange future visits. The investigator participated in an exercise session with the volunteer at the weekly meetings. This meeting allowed the investigator to supervise an exercise session and verify the volunteer's ability to participate in an exercise session for the given duration and at the given workload. The weekly meetings also maintained the focus

and motivation of the volunteer. Each session was analysed for duration and mean heart rate achieved.

2.7.2 Exercise Activities and Facilities

Volunteers were given free access to the Sports and Recreation Service facilities at Glasgow University for the duration of their involvement with the study. These facilities provided a focal point for exercise sessions and weekly meetings, and allowed volunteers access to a wide variety of exercise equipment, training areas, swimming pool and the opportunity to participate in organised classes. Volunteers were allowed to use whichever cardiovascular exercise equipment they preferred (e.g stepper, treadmill, cycle ergometer, rowing ergometer) or attend scheduled aerobic exercise classes. Alternatively, they were allowed to run, cycle or perform other modes of exercise at other locations, provided they completed the required duration and intensity of exercise.

To assist volunteers in planning their next week of exercise, an exercise planner (Appendix B2) was provided at each weekly meeting. The planner contained a reminder of their target heart rate, number and duration of sessions, and timing of the next weekly meeting. The planner also provided a diary facility to allow the volunteer to record the time of each session, and the exercise performed.

2.8 Dietary Monitoring and Analysis

Participants were instructed to keep a food diary (Appendix B3) for seven consecutive days which involved weighing all food and drink on electronic scales (Salter Housewares, Kent, United Kingdom) and recording the weight and time of consumption in the diary. Diaries were completed prior to each metabolic test. Instructions were provided in addition to a

visual demonstration by the researcher to show how to use the scales and the diary. The participants were advised to maintain their normal dietary intake. Volunteers were encouraged to weigh constituents of each meal and record the details of the food type and weight of food used. If a meal was not completely consumed, they were asked to weigh and record the remaining food constituents to allow investigators to calculate accurate consumption. In circumstances where it was not possible to weigh constituent components of a meal (e.g. a pre-packed sandwich), the volunteers were asked to record the weight of the total meal and provide a description of the meal. CompEat 5.0 (Nutrition Systems, Banbury, United Kingdom) was then used to analyse the food diaries and determine macronutrient and micronutrient intake for each subject.

2.9 Evaluation of habitual physical activity

For the seven day period prior to each metabolic test, volunteers participated in physical activity recording. Volunteers completed an activity diary (Appendix B4) during this period, and were asked to record all activities performed during waking hours. Detailed description of activities was not required, however the type of activity and duration was requested. Activity diaries are frequently used as the criterion measure in the validation of physical activity questionnaires (Pols et al. 1996) and appear to be a reliable method of performing repeated measurements of physical activity (Bouchard et al. 1983). Additionally, for waking hours, volunteers were asked to wear their heart rate monitors. The memory storage facility of the monitors was used to record a mean heart rate for each minute worn.

2.9.1 Heart Rate Recording

Continuous daily heart rate recording provided a measurement of habitual physical activity. Activity diaries served as a validation tool for the accuracy of the heart rates recorded, and allowed the use of an estimated heart rate (based on the recorded activity) for periods where

heart rate data was not successfully recorded. For example, close proximity to devices producing electromagnetic fields (e.g. laptop fans, induction loops) caused interference which served to cause a recorded heart rate of 230 beat.min⁻¹. Activity diaries were useful in these situations to allow the use of an appropriate estimated heart rate for these periods.

2.9.2 Evaluation of energy expenditure of exercise sessions

For each exercise training session the mean heart rate of the session was converted into an equivalent $\dot{V}O_2$ based on the individualised heart rate versus $\dot{V}O_2$ relationship. The individual's $\dot{V}O_2$ associated with sedentary activity was subtracted from this value to determine the net exercise $\dot{V}O_2$ (i.e. the oxygen cost of exercise over and above that of sedentary daily activities). The net exercise $\dot{V}O_2$ (l.min⁻¹) was then multiplied by the duration of exercise to determine the total net oxygen cost of the exercise session, and the net energy cost of the session (in kJ) was obtained by multiplying the net oxygen cost by 20.3 (Frayn & Macdonald I.A. 1997). For exercise sessions performed during the first 3.5 weeks of the programme, energy expenditure values were determined using the heart rate versus $\dot{V}O_2$ relationship from the baseline fitness test; for sessions during the second 3.5 weeks of the programme, values obtained during the post-intervention fitness test were used in the energy expenditure calculations. The net energy expenditure of all exercise sessions was summed to determine the total net energy expenditure of the exercise training programme.

2.9.3 FLEX Pilot Study

The 'FLEX' method of energy expenditure calculation utilises free-living measurements of minute-by-minute heart rates to estimate total daily energy expenditure (Ceesay et al. 1989; Spurr et al 1988; Spurr 1990). This method compares well (Spurr et al. 1988; Livingstone et al. 1990; Leonard 2003) with the 'gold-standard' method of energy expenditure calculation using doubly-labelled water (Ainslie, Reilly, & Westerterp 2003; Klein et al. 1984). The FLEX method is less expensive and less technically complicated than the doubly-labelled water method (Schoeller & Webb 1984; Spurr et al. 1988) and takes into account the loss of the linear relationship between heart rate and $\dot{V}O_2$ (and thus energy expenditure) at low intensity heart rates (Montoye HJ 1996) (Figure 2.9).

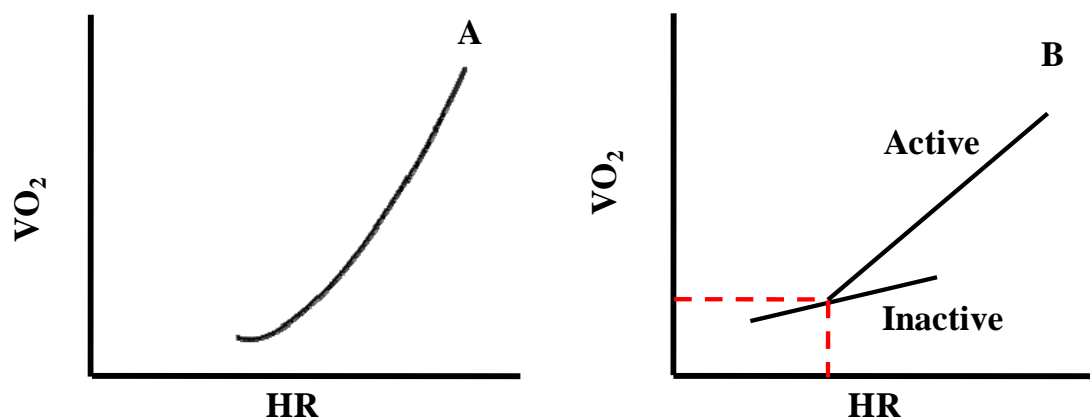


Figure 2.9. **A** Oxygen consumption determined by heart rate³ showing non-linear relationship. **B** Separation of 'active' and 'non-active' heart rates by observed activity, and corresponding determination of threshold above which linear relationship between HR and $\dot{V}O_2$ applies, and below which a non-linear relationship exists. Dotted red line indicates inflexion point (FLEX) between active and inactive heart rates.

Determination of the FLEX point requires accurate measurement of heart rates at rest and during sedentary activities. It was observed that the difference in resting heart rate before and after the exercise intervention was considerably greater when determined during the FLEX

measurements (i.e. immediately prior to exercise, as described in section 2.5.1), than when measured using the Complior method (COMP), or from the lowest heart rate values recorded during the Continuous Heart Rate Monitoring weeks (CHRM) (Figure 2.10). This discrepancy between pre- and post-intervention FLEX measurements was greater than would have been expected as a results of improved cardiorespiratory fitness, and therefore questioned the accuracy of the heart rates measured during determination of the FLEX point.

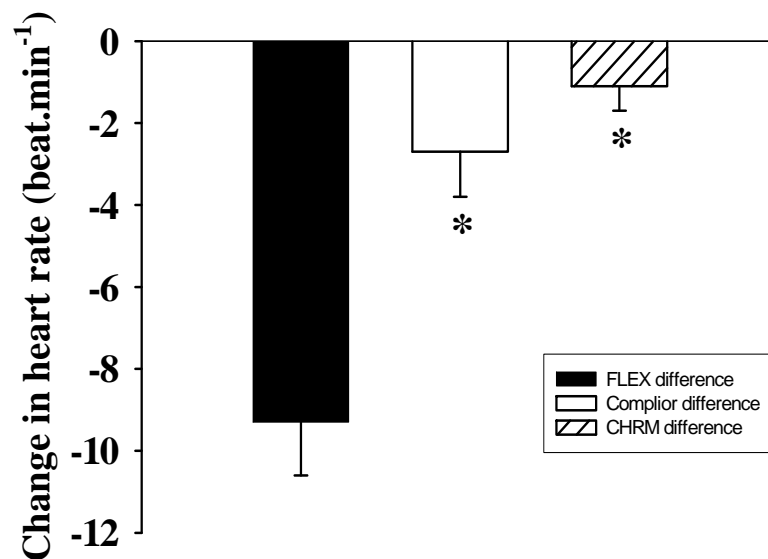


Figure 2.10. Difference between pre- and post-intervention resting heart rate measured by: FLEX measurements, Complior and Continuous Heart Rate Monitoring (CHRM). * $p < 0.001$ compared with change by FLEX measurement.

2.9.3.1 Methodology for the evaluation of the FLEX Method

To determine the impact of a possible exaggerated difference in pre- and post-intervention FLEX heart rate values on calculated energy expenditure, a pilot study was performed using the data from the first fifteen volunteers to complete pre- and post-intervention testing. The FLEX method defined a heart rate threshold (the ‘FLEX’ point) above which was categorised as ‘active’, and below which was categorised as ‘inactive’. For ‘active’ heart rates, the

relationship between heart rate and oxygen consumption was assumed to be valid, and therefore active energy expenditure (Active EE) was calculated based on an individual's oxygen consumption and heart-rate relationship derived from an exercise test (Section 2.6). For sleep periods, energy expenditure was given as equivalent to resting metabolic rate (RMR) (Section 2.3.2). For waking periods at \leq FLEX, so-called 'sedentary energy expenditure' was a mean value calculated as the average energy expenditure for the sedentary stages (lying down, sitting, standing) during an active-inactive calibration (FLEX EE) (Ceesay et al. 1989; Spurr et al. 1988). The FLEX point was an individually determined heart rate which was defined as the average of the highest resting heart rate, and lowest exercise heart rate recorded during the assessment of heart rate and $\dot{V}O_2$ during rest and exercise (Section 2.8 – 2.9) (Ceesay et al. 1989; Spurr et al. 1988; Spurr 1990).

Energy expenditure was calculated using the equations of indirect calorimetry (section 2.3.3), where total daily energy expenditure was composed of:

1. Sleeping energy expenditure ((1440 – number of minutes recorded) x RMR)
2. Sedentary energy expenditure (number of minutes \leq FLEX) x FLEX EE
3. Active energy expenditure (number of minutes $>$ FLEX) x Active EE

To adjust for the observed difference in resting heart rates, energy expenditure was re-calculated using a variety of adjusted FLEX heart rates. Pre-intervention values of daily energy expenditure were compared to post-intervention values calculated using:

1. Post-intervention FLEX heart rate derived at post-intervention re-assessment.
2. Pre-intervention FLEX heart rate (FLEX 1).
3. Pre-intervention FLEX heart rate adjusted by the difference between pre- and post-intervention resting heart rate as calculated by continuous heart rate monitoring (FLEX CHRM).

4. Pre-intervention FLEX heart rate adjusted by the difference between pre- and post-intervention resting heart rate as calculated by Complior measurement. (FLEX COMP).

2.9.3.2 Results

By using the post-intervention values derived by the FLEX method, and after adjustment for the energy cost of the exercise sessions in the monitoring week, a significant increase in energy expenditure of $321.9 \pm 107.6 \text{ kcal.day}^{-1}$ was noted ($p < 0.01$). However, using adjusted values of FLEX did not reveal any difference in habitual energy expenditure (Table 2.2).

Table 2.2 Estimated total daily energy expenditure at baseline and post-intervention.

Method of EE assessment	Value (kcal.day ⁻¹)	p-value
Pre-Intervention FLEX EE	2396	-
Post-Intervention FLEX EE	2718	< 0.01
Post-Intervention FLEX 1	2409	NS
Post-Intervention FLEX COMP	2501	NS
Post-Intervention FLEX CHRM	2462	NS

Post-intervention values adjusted using baseline FLEX point (FLEX 1), pre-intervention FLEX heart rates adjusted as described. P-values calculated comparing pre-intervention FLEX EE with post-intervention values using repeated measures ANOVA with Tukey HSD *post-hoc* analysis.

2.9.3.3 Discussion

In summary, discrepancies between those resting heart rate measurements made during the active-inactive calibration and those made during both the Complior measurements and CHRM recordings suggested that the calculated FLEX heart rates might be incorrect. Resting heart rates measured using the CHRM method are representative of free-living values and Complior resting heart rate is measured after a rest period of 30 minutes in an environment with minimal external stimuli, these are likely to be more accurate measurements of true resting heart rate. This was borne out by the significant difference observed between calculated energy expenditure pre- and post-intervention using the FLEX heart rates, which was not observed using heart rates which were adjusted for more realistic and physiological changes in resting heart rates.

It is unclear why there were such differences in resting heart rates measured during the determination of the relationships between heart rate and $\dot{V}O_2$ during rest and exercise. The FLEX measurements were amongst the first tests in which volunteers participated. The initial exposure to a new laboratory environment, and to unfamiliar technical staff and procedures could have been a more stressful experience than anticipated, leading to falsely elevated resting heart rates at this time. This pilot study suggests that in this case the FLEX heart rates calculated prior to, or after the exercise intervention (or both) were not accurate representations of the 'true' FLEX point(s) and subsequently estimated energy expenditure values were not deemed to be valid. It was therefore decided not to use the FLEX method to determine energy expenditure in this study. Further attempts to use the FLEX method to determine energy expenditure may require volunteers to be 'familiarised' with the laboratory environment and for resting heart rate measurements to be validated against established methods (Voors, Webber, & Berenson 1982).

2.10 Indices of physical activity

In view of the difficulties with the FLEX method, an alternative index of habitual activity was used. The problems with the FLEX technique were apparent only after the majority of the study had been conducted and there was no opportunity to design an alternative method of calculating habitual energy expenditure. Instead, indices of physical activity based on heart rate observed during the continuous seven day monitoring period were used. Average daily heart rate was calculated from heart rate data obtained during the waking hours. Additionally, resting heart rate was determined as the lowest heart rate recorded during waking hours.

Indices of physical activity were determined by calculating the number of minutes spent at multiples of resting heart rate. Time spent at heart rates greater than resting multiplied by 1.5 and 2 were used as markers of light and moderate physical activity, respectively. Thus, given a typical resting heart rate of $70 \text{ beat} \cdot \text{min}^{-1}$, light activity would be equivalent to a heart rate of around $105 \text{ beat} \cdot \text{min}^{-1}$, and moderate activity at a heart rate of around $140 \text{ beat} \cdot \text{min}^{-1}$.

Average daily heart rate minus resting heart rate (i.e. the time averaged area under the heart rate versus time curve, using resting heart rate as baseline) was used as a surrogate measure of total activity.

2.11 Power Calculation

Based on reports in the literature, the within-subject reproducibility coefficient of variation for OGTT-assessed insulin resistance is approximately 8% (Mari et al. 2001); the standard deviation for between-subject variation in insulin resistance in normoglycaemic individuals is approximately 25-40% (Lihn et al. 2003; Perseghin et al. 1997); offspring of patients with type 2 diabetes are approximately 40-50% more insulin resistant than matched control subjects (Higgins et al. 2005; Humphriss et al. 1997; Lihn et al. 2003) and the planned exercise intervention would be expected to improve insulin sensitivity by approximately 20-30% (Borghouts & Keizer 2000). Assuming the between-subject standard deviation for insulin resistance is 40%, 80 volunteers (40 control, 40 offspring) was expected to enable detection of a 25% difference in insulin resistance between the control and offspring groups at $\alpha = 0.05$

and 80% power. Previous studies have shown that this number of volunteers is sufficient to show a difference in insulin resistance between control and offspring subjects at the $p = 0.001$ level (Higgins et al. 2005). Twenty subjects in each group (Offspring Exercise, Offspring Delayed Exercise, Control Exercise, Control Delayed Exercise) was expected to allow detection of a 5% change in insulin resistance with exercise and a 5% difference in the change in insulin resistance with exercise between the control and offspring groups with the same power.

2.11.1 Statistical analysis

All statistical analysis were performed using Statistica (version 6.0, StatSoft Inc., Tulsa, Oklahoma) and Minitab (version 13.1, Minitab Inc., State College, Pennsylvania). Differences between the Offspring and Control groups at baseline were assessed by unpaired t-tests. Exercise-induced effects were assessed by comparison of the change in variables (i.e. post-intervention minus pre-intervention) among the groups by 2-way ANOVA (family history x intervention) with *post-hoc* Tukey tests. Determinants of change in insulin sensitivity were assessed by univariate linear correlations. Multiple regression analyses were performed to establish the independence of relationships. Anderson-Darling normality testing was performed on all data sets, and logarithmic transformation was performed (where necessary), prior to analysis.

2.11.2 Index of Insulin Sensitivity calculation

In all experimental chapters insulin sensitivity was measured using the Insulin Sensitivity Index (ISI) devised by Matsuda and DeFronzo (1999). This utilises the glucose and insulin values for each time point of an oral glucose tolerance test and is highly correlated with the rate of whole-body glucose disposal during a clamp (Matsuda & DeFronzo 1999). The ISI was calculated using equation 2.17.

$$\text{ISI} = 10000 / \sqrt{[(\text{Fasting Glucose} \times \text{Fasting Insulin}) \times (\text{Mean Glucose during OGTT} \times \text{Mean Insulin during OGTT})]} \quad \textbf{Equation 2.17}$$

Mean glucose and insulin concentrations during OGTT were calculated as the average of the 30, 60, 90 and 120 minute values. For the purposes of equation 2.17, glucose was measured in mg.dl⁻¹ and insulin in µU.ml⁻¹. Based on data from the two baseline measurements made in the volunteers randomised to the ‘Delayed Exercise’ arm, the within-participant test-retest coefficient of variation for ISI was 12.2%, with mean values for ISI differing by 3.2% between the two baseline measurements.

CHAPTER 3

PHYSICAL AND METABOLIC CHARACTERISTICS OF SEDENTARY WOMEN WITH AND WITHOUT A FAMILY HISTORY OF TYPE 2 DIABETES

3.1 Introduction

First degree relatives of patients with type 2 diabetes have a greater risk of developing diabetes than their counterparts with no family history of diabetes (Kobberling J & Tillil H 1982;Ohlson et al. 1988). Insulin resistance is the best risk predictor for the development of diabetes (Ferrannini 1998;Laws & Reaven 1993), which is consistent with the observation that healthy first degree relatives of type 2 diabetic patients (relatives) are often more insulin resistant than individuals without a family history of diabetes (controls) (Nyholm et al. 2004;Perseghin et al. 1997;Tesauro et al. 2007;Higgins et al. 2005;Ahn et al. 2004;Humphriss et al. 1997;Nyholm et al. 1996;Ostergard et al. 2006;Johanson et al. 2003). In some of the studies (Humphriss et al. 1997;Higgins et al. 2005) impaired insulin sensitivity observed in relatives could be explained by higher measures of adiposity, a central contributing factor to insulin sensitivity (Chang et al. 2004;Salmenniemi et al. 2005;Kriketos et al. 2004). However in other studies the differences in insulin sensitivity were evident despite no difference in body mass or total body fat between relative and control groups (Nyholm et al. 1997;Johanson et al. 2003;Nyholm et al. 2004;Ostergard et al. 2006). Similar observations have been made in other groups (e.g. South Asians, American Pima Indians) who display an increased predisposition to type 2 diabetes in comparison to Caucasians with comparable adiposity (McCance et al. 1994;Razak et al. 2007;Chandalia et al. 2007). This suggests that, similar to these other groups, insulin sensitivity in relatives of patients with type 2 diabetes may be influenced by adiposity to a greater degree than in individuals without family history of diabetes. One aim of the current study was therefore to explore the influences of adiposity on insulin sensitivity in this group, using a robust assessment of adiposity.

In addition to differences in adipose tissue mass and distribution, relatives of patients with type 2 diabetes have been reported to have altered adipose tissue function, characterised by higher circulating NEFA (Perseghin et al. 1997; Lattuada et al. 2005) and pro-inflammatory cytokines such as IL-6, TNF α (Maltezos et al. 2002; Straczkowski et al. 2002) and reduced concentrations of insulin-sensitising cytokines such as adiponectin (Lattuada et al. 2005; Tesauro et al. 2007). In the presence of insulin resistance, altered vascular endothelial function has been observed (Scuteri et al. 2008; Tesauro et al. 2007; McEleavy et al. 2004), which may predispose to premature cardiovascular disease. A further aim of this study was therefore to explore the interaction between adipose tissue function, vascular function and insulin resistance using measurements of circulating adipokines as a proxy measure of adipose tissue function and Pulse Wave Velocity (PWV) as a marker of vascular function.

There is some evidence to suggest that both physical activity and diet play an important role in determining differences in insulin sensitivity seen between those with and without a family history of T2D. For example, it has been shown that early metabolic abnormalities of offspring of type 2 diabetic patients are partially ameliorated by participation in regular aerobic exercise (Ahn et al. 2004). In addition, Higgins and colleagues (2005) reported lower levels of habitual physical activity in relative subjects compared to control subjects and observed a stronger relationship between physical activity and insulin sensitivity in the relative group (Higgins et al. 2005). However, as Higgins and colleagues used self-report measures for the physical activity assessment, which have limited reliability (Klesges et al. 1990), and assessed insulin resistance by HOMA, using fasting glucose and insulin concentrations only (Matthews et al. 1985), the data may not be entirely robust and require confirmation. Using more robust measurements of both insulin sensitivity and habitual activity, the current study aimed to investigate the notion that insulin sensitivity in relatives may be modulated to a greater degree by physical activity, than in control volunteers.

To date only three studies have made an attempt to investigate differences in dietary intake between relatives and control individuals (Adamson et al. 2001; Johanson et al. 2003; Higgins et al. 2005), and only two studies have investigated the role of diet in determining differences in insulin sensitivity in these groups (Johanson et al. 2003; Higgins et al. 2005). Two existing studies reported that there were no significant differences in daily energy and macronutrient intake or the proportion of energy provided by the main nutrients between relative and control groups (Higgins et al. 2005; Johanson et al. 2003). Furthermore, Higgins and colleagues (2005) showed that neither the control nor the relative group's dietary factors correlated with insulin sensitivity (Higgins et al. 2005). Contrary to this, Adamson and co-workers (2001) observed that non-diabetic relatives of type 2 diabetic patients consumed diets which are expected to promote the development of diabetes (Adamson et al. 2001). Indeed, in this study relatives were reported to have higher absolute intakes of total fat, saturated fat and cholesterol and lower intake of non-starch polysaccharides (Adamson et al. 2001). In both studies, relatives were heavier than controls, therefore the current study aimed to examine the role of dietary factors in determining insulin sensitivity in offspring of people with type 2 diabetes, and matched controls.

Therefore, aims of this chapter were: firstly, to determine differences in the metabolic phenotype between sedentary females with and without a family history of type 2 diabetes; secondly, to explore the interaction between adipose tissue function, inflammation and both insulin resistance and vascular function in this group; additionally, to determine the extent by which adiposity and lifestyle factors, such as physical activity, contribute to the insulin resistant phenotype observed in those with a family history of diabetes; finally, to ascertain whether effects of adiposity and lifestyle factors on insulin sensitivity differ between those with, and without a family history of the disease.

3.2 Methods

3.2.1 Volunteers

Volunteers were recruited and screened as detailed in section 2.1. Specific inclusion criteria are defined in section 2.1.3. Further information detailing recruitment response and excluded volunteers can be found in Figure 2.2. The Offspring group was composed of women with a first-degree relative with type 2 diabetes. The Control group was composed of women with no first or second degree relative with type 2 diabetes. This study was conducted on thirty four Offspring and thirty six Control volunteers. Control and Offspring groups were well matched for age (Controls 33.6 ± 6.1 years, Offspring 35.6 ± 7.0 years, $p = 0.20$) and BMI (Controls 27.3 ± 4.7 kg.m⁻², Offspring 28.1 ± 5.1 kg.m⁻², $p = 0.50$).

3.2.2. Study Design

All participants underwent assessment of physical characteristics, body composition, cardiorespiratory fitness and participated in metabolic testing as detailed in Sections 2.2-2.3 and 2.6 respectively. They were also asked to complete a diary of habitual physical activity and food intake. In addition they were asked to participate in continuous waking hours heart rate monitoring prior to which the individual relationships of heart rate to $\dot{V}O_2$, and $\dot{V}CO_2$ under resting and exercise conditions were established.

3.2.3 Energy and macronutrient intake

For a seven day period prior to metabolic investigation, volunteers were asked to keep a record of a food diary for seven consecutive days as described in Section 2.8.

3.2.4 Establishment of relationships of heart rate to $\dot{V}O_2$, and $\dot{V}CO_2$

Individual relationships between heart rate, $\dot{V}O_2$ and $\dot{V}CO_2$ were established during both rest and exercise as described in Sections 2.5 and 2.6 respectively.

3.2.5 Evaluation of habitual physical activity

Heart rate measurement throughout the day (as described in section 2.6.1) was used to assess indices of habitual physical activity (Section 2.10). Assessment of time spent at heart rates which were 1.5 and 2 times greater than resting heart rate were considered to be represent approximate thresholds for light and moderate activity respectively (Section 2.10). Average daily heart rate minus resting heart rate was used as a surrogate measure of total activity (Section 2.10).

3.2.6 Evaluation of cardiorespiratory fitness

Cardiorespiratory fitness was measured by predicted $\dot{V}O_{2max}$ and lactate threshold, as described in section 2.6.

3.2.7 Anthropometric assessment

Measurements of body mass, height, skinfold thickness, waist and hip circumference were made as described in Section 2.2. Additional assessment of body composition was made using DEXA scanning, as described in Section 2.2.5.

3.2.8 Metabolic testing

As described in Section 2.3, volunteers attended the Metabolic Investigation Suites at the Institute of Diet, Exercise and Lifestyle (IDEAL) after a 12-hour overnight fast. Volunteers had fasting measurements of resting metabolic rate, substrate utilisation and peripheral PWV (see Sections 2.3.2, 2.3.5 respectively) which was followed by fasting blood sampling (Section 2.3.6). After fasting samples were taken, the volunteers proceeded to subcutaneous abdominal adipose tissue liposuction biopsy (Appendix B1, data not presented in this thesis). On completion of the biopsy process, volunteers commenced a 75 g oral glucose tolerance test (section 2.3.7) with further blood samples were taken at 30, 60, 90 and 120 minutes after glucose ingestion. Samples were processed and analysed as described in Section 2.4. Upon completion of the glucose tolerance test, the volunteers had repeat PWV assessment and measurements of substrate utilisation and metabolic rate (Figure 2.5).

3.2.9 Blood analysis

After centrifugation all plasma samples were stored at -80°C in labelled 2 ml Eppendorf tubes (Alpha Laboratories, Hampshire, UK). Glucose, NEFA, 3-OHB, ALT, GGT, total cholesterol, HDL-cholesterol, TG and CRP were all measured by spectrophotometric techniques as described in Section 2.4.2. LDL-cholesterol was calculated by the Friedewald equation (Friedewald, Levy, & Fredrickson 1972). Insulin, adiponectin, resistin, leptin, TNF α , IL-6 were measured by ELISA technique as described in section 2.4.3.

3.2.9.1 Calculation of glucose, insulin, NEFA, and 3-OHB concentrations and insulin sensitivity during oral glucose tolerance test

The mean concentration of glucose, insulin, NEFA and 3-OHB during the oral glucose tolerance test (OGTT) was calculated by dividing the area under variable *vs.* time curve (total AUC) by the duration of the OGTT (mean during OGTT). The rise (or suppression) of glucose, insulin, NEFA and 3-OHB was calculated by subtracting the area under the fasting concentration *vs.* time curve from the total AUC and dividing the obtained value by the duration of the OGTT (rise during OGTT). For variables where values decreased from baseline after glucose ingestion (e.g. NEFA), the rise during OGTT was negative and represented the area above the curve extending up to the baseline value, providing an index of post-glucose suppression. Insulin sensitivity was calculated using the Insulin Sensitivity Index (ISI) as described by Matsuda and DeFronzo (Matsuda & DeFronzo 1999). This equation is outlined in section 2.11.2.

3.2.9.2 Statistical analysis

Data were analysed using Statistica (version 6.0, StatSoft Inc., Tulsa, Oklahoma) and Minitab (version 13.1, Minitab Inc., State College, Pennsylvania). Prior to analysis, all data were tested for normality using the Anderson-Darling normality test and, if necessary, logarithmically transformed. All data between Offspring and Control groups was compared using unpaired t-tests. Univariate linear regressions were performed, using logarithmically transformed data where required. Correlations between ISI and other variables were performed for the Offspring and Control groups. To determine the extent to which variables were related to ISI independently of fat mass, univariate linear regressions were then performed between the residuals for ISI of the regression between ISI and total fat mass. This classic statistical approach has been used previously to determine the independent effects of individual variables within multi-factorial systems on biological outcomes (Speakman et al. 2002; Westerterp & Speakman 2008) and, in effect, provides the correlations between ISI and

other variables, adjusted for the effect of total fat mass. Significance was accepted at $p \leq 0.05$.

3.3 Results

3.3.1 Concentrations of glucose, insulin, non-esterified fatty acid and 3-hydroxybutyrate

Fasting concentrations of plasma glucose, insulin, non-esterified fatty acid (NEFA) and 3-hydroxybutyrate (3-OHB) are presented in Table 3.1 and Figure 3.1. No significant differences between Controls and Offspring were found for glucose, NEFA or 3-OHB but Offspring had significantly higher fasting insulin concentrations compared to Controls (Table 3.1, Figure 3.1). Mean concentrations of glucose, insulin, NEFA and 3-OHB during the OGTT are presented in Table 3.1 and Figure 3.1. Mean concentration of glucose, insulin and NEFA were not significantly different between groups whilst mean concentration of 3-OHB was significantly higher ($p < 0.01$) in Offspring compared to Controls. Mean change of glucose, insulin, NEFA and 3-OHB concentration during OGTT did not differ between Control and Offspring groups.

Table 3.1 Glucose, insulin, non-esterified fatty acid (NEFA) and 3-hydroxybutyrate (3-OHB) concentrations in the fasted state and during OGTT. Values are mean \pm SEM.

	Controls	Offspring	P - value
Glucose			
Fasting (mmol.l ⁻¹)	4.8 \pm 0.5	4.9 \pm 0.7	0.52
Mean during OGTT (mmol.l ⁻¹)	6.4 \pm 1.3	7.0 \pm 1.5	0.09
Change from fasting during OGTT (mmol.l ⁻¹)	1.6 \pm 0.2	2.1 \pm 0.2	0.11
Insulin			
Fasting ^a (mU. l ⁻¹)	6.2 \pm 3.8	7.9 \pm 4.5	< 0.05
Mean during OGTT ^a (mU. l ⁻¹)	50.4 \pm 35.5	61.8 \pm 38.9	0.11
Change from fasting during OGTT ^a (mU.l ⁻¹)	44.2 \pm 5.4	53.9 \pm 6.0	0.12
NEFA			
Fasting (mmol.l ⁻¹)	0.52 \pm 0.18	0.55 \pm 0.17	0.39
Mean during OGTT ^a (mmol.l ⁻¹)	0.25 \pm 0.11	0.26 \pm 0.07	0.28
Change from fasting during OGTT (mmol.l ⁻¹)	-0.27 \pm 0.02	-0.29 \pm 0.02	0.41
3-OHB			
Fasting ^a (mmol.l ⁻¹)	0.06 \pm 0.05	0.09 \pm 0.07	0.16
Mean during OGTT (mmol.l ⁻¹)	0.04 \pm 0.004	0.07 \pm 0.05	< 0.01
Change from fasting during OGTT (mmol.l ⁻¹)	-0.02 \pm 0.01	-0.01 \pm 0.01	0.11

P-values are for unpaired *t*-tests between groups. ^astatistical analysis performed on logarithmically transformed data.

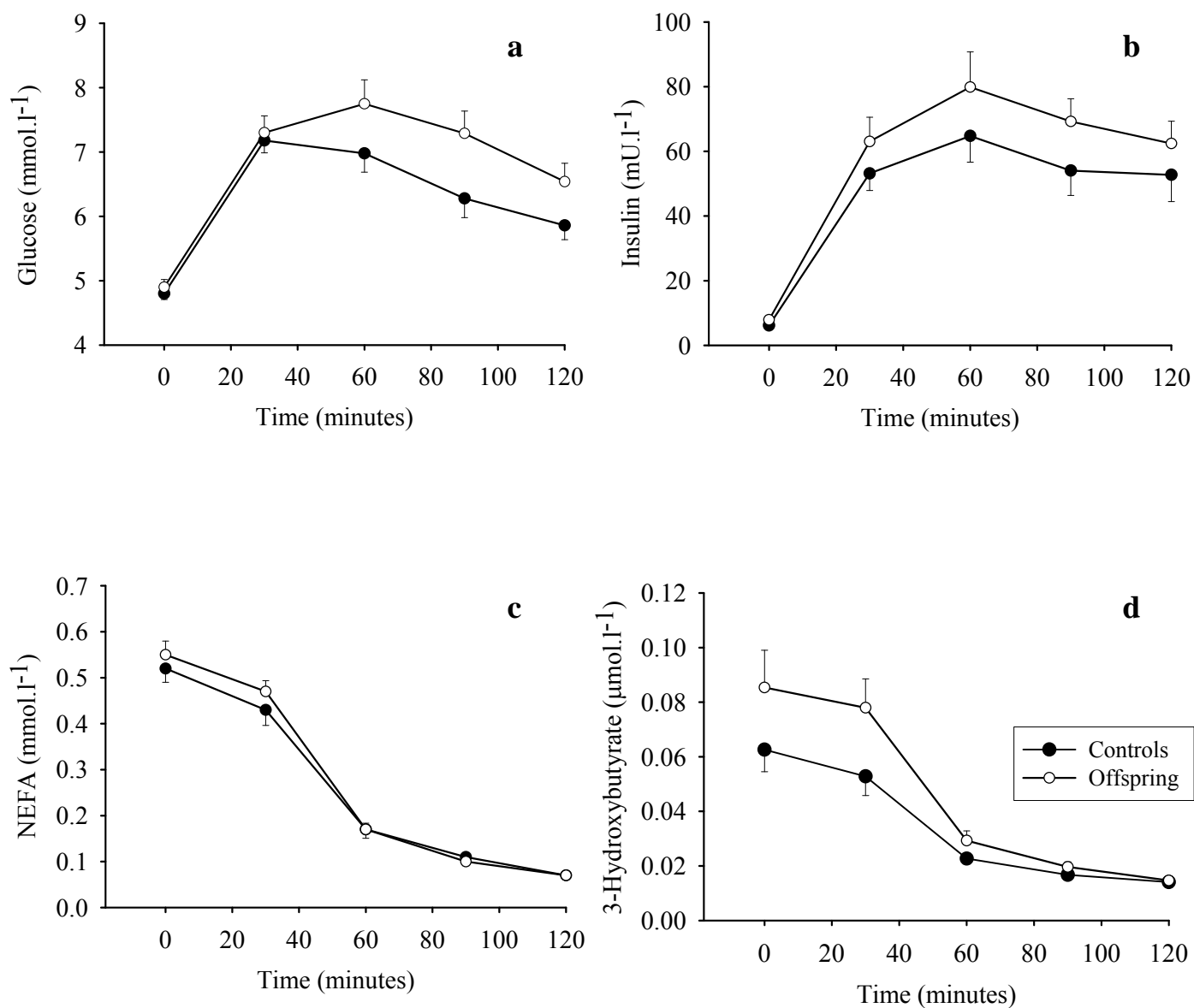


Figure 3.1 Plasma glucose (a), insulin (b), NEFA (c) and 3-OHB (d) concentrations for Offspring and Control in the fasting state and during the OGTT. Values are mean \pm SEM.

3.3.2 Insulin sensitivity

The Insulin Sensitivity Index (ISI) (Matsuda & DeFronzo 1999) of Offspring was 22% lower ($p = 0.047$) than Controls (Figure 3.2).

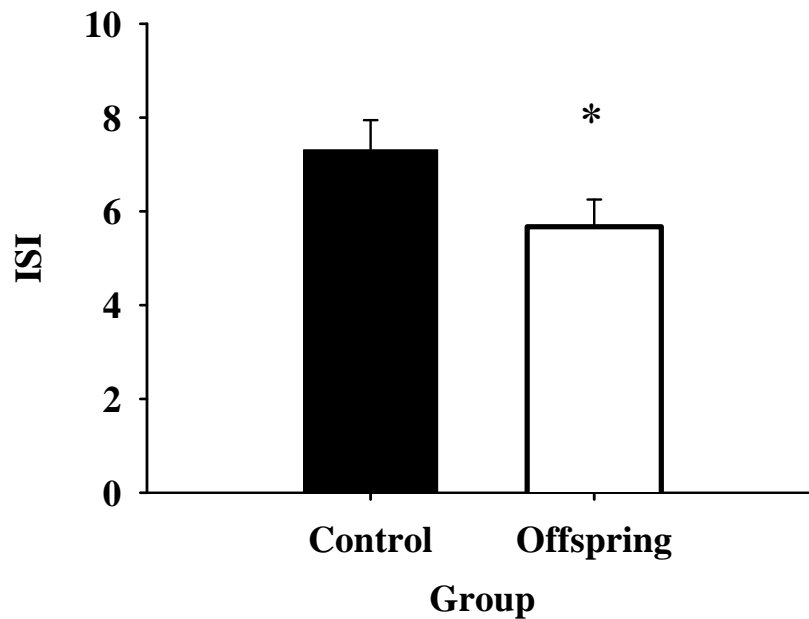


Figure 3.2 ISI in Control and Offspring. Values are Mean \pm SEM. P-value is for unpaired t -tests between groups. *significantly different ($p < 0.05$) from Control group.

Fasting concentrations of non-esterified fatty acids (NEFA) and 3-hydroxybutyrate (3-OHB) were not correlated with ISI in either group, either before or after adjustment for fat mass. In Offspring, mean concentration of NEFA during the OGTT was significantly negatively associated with ISI ($r = -0.42$, $p < 0.05$), but only after adjustment for the effects of fat mass.

3.3.3 Physical characteristics and body composition

There were no differences between groups for any index of body composition (Table 3.2).

Table 3.2 Physical characteristics and body composition of Control and Offspring.

	Controls	Offspring	P - value
	(n=36)	(n=34)	
Body mass (kg)	73.0 ± 14.8	76.6 ± 15.1	0.33
Total fat mass (kg)	28.9 ± 10.1	31.3 ± 10.2	0.33
Trunk fat mass (kg) ^a	14.2 ± 5.3	16.3 ± 5.6	0.13
Leg fat mass (kg) ^a	10.0 ± 3.2	10.3 ± 3.2	0.76
Arm fat mass (kg) ^a	3.6 ± 1.6	3.9 ± 1.9	0.58
Android fat mass (kg)	2.3 ± 1.0	2.7 ± 1.2	0.12
Gynoid fat mass (kg)	5.2 ± 1.4	5.5 ± 1.3	0.91
Total lean mass (kg)	42.1 ± 5.6	42.6 ± 6.0	0.69
Trunk lean mass (kg)	20.9 ± 3.0	21.3 ± 3.2	0.59
Leg lean mass (kg)	13.8 ± 2.1	14.0 ± 2.3	0.64
Arm lean mass (kg) ^a	4.2 ± 0.8	4.4 ± 0.9	0.39
Upper Body Fat (kg) ^a	17.7 ± 6.8	20.2 ± 7.3	0.18
Lower Body Fat (kg) ^a	10.0 ± 3.2	10.3 ± 3.2	0.76
Waist Circ (cm) ^a	84.4 ± 11.6	89.1 ± 13.1	0.12
Hip Circ (cm) ^a	106.7 ± 9.7	109.0 ± 10.1	0.33
Biceps Skinfold (mm) ^a	14.7 ± 5.7	16.8 ± 8.3	0.33
Triceps Skinfold (mm) ^a	24.0 ± 6.6	23.5 ± 7.3	0.55
Subscapular Skinfold (mm)	23.2 ± 8.9	23.7 ± 7.1	0.78
Suprailiac Skinfold (mm) ^a	23.9 ± 8.0	27.0 ± 9.4	0.12

Values are Mean ± SD. P-values are for unpaired *t*-tests between groups. ^astatistical analysis performed on logarithmically transformed data.

Total fat mass was significantly negatively correlated with ISI in both Controls and Offspring and correlated more strongly with ISI in Offspring than Controls (Figure 3.3). In Offspring alone lean mass was also negatively correlated with ISI, ($r = -0.614$, $p < 0.001$) with consistent negative association in all regional measurements of lean mass (trunk: $r = -0.601$, $p < 0.001$; leg: $r = -0.534$, $p < 0.01$; arm: $r = -0.650$, $p < 0.001$). In the Control group, most indices of adiposity were negatively associated with ISI (Table 3.3). Offspring showed stronger and more consistent association between adiposity and ISI than Controls, exhibiting significant negative correlation with all indices of adiposity. After adjustment for the influence of fat mass, neither Controls nor Offspring showed any significant correlation between indices of adiposity and ISI.

Figure 3.3 Scattergrams illustrating the correlation of fat mass with ln ISI in Control and Offspring groups.

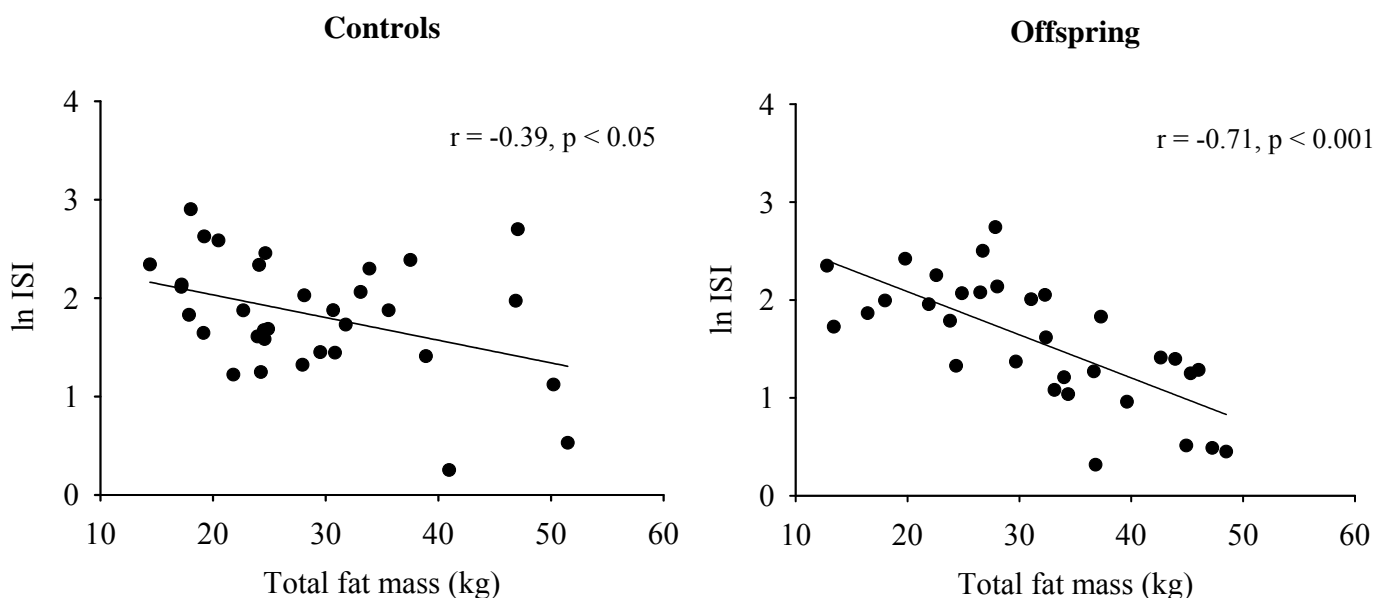


Table 3.3 Correlations between ISI and indices of adiposity for Control (n = 36) and Offspring (n = 34) groups.

		Controls		Offspring	
		Unadjusted	Adjusted	Unadjusted	Adjusted
Biceps skinfold ^a	r	-0.31	0.12	-0.54^{**}	-0.14
Triceps skinfold ^a	r	-0.26	0.23	-0.45^{**}	-0.09
Subscapular skinfold	r	-0.54^{***}	-0.14	-0.45^{**}	-0.02
Suprailiac skinfold ^a	r	-0.45^{**}	-0.03	-0.61^{***}	-0.28
Waist – hip ratio	r	-0.51^{**}	-0.23	-0.58^{***}	-0.27
BMI	r	-0.37[*]	0.23	-0.72^{***}	-0.26
Waist circumference ^a	r	-0.50^{**}	0.05	-0.65^{***}	-0.23
Hip circumference ^a	r	-0.32	0.26	-0.62^{***}	-0.17
Trunk fat mass ^a	r	-0.45^{**}	0.15	-0.72^{***}	-0.27
Leg fat mass ^a	r	-0.30	0.30	-0.58^{***}	-0.12
Arm fat mass ^a	r	-0.39[*]	0.20	-0.62^{***}	-0.14
Upper body fat ^a	r	-0.44^{**}	0.16	-0.72^{***}	-0.24
Lower body fat ^a	r	-0.30	0.30	-0.58^{***}	-0.12

r = Univariate correlations of variable vs. ISI. Unadjusted = variable correlated against ISI.

Adjusted = variable correlated against residual of ISI and fat mass. ^astatistical analysis

performed on logarithmically transformed data. Statistically significant correlation shown in

bold. * p < 0.05, ** p < 0.01, *** p < 0.001.

3.3.4 Metabolic rate & substrate utilisation

No differences were observed between Offspring and Controls in resting metabolic rate (RMR), respiratory exchange ratio (RER), or rates of fat and carbohydrate oxidation measured in the fasting state (Table 3.4). Furthermore, no significant differences between Controls and Offspring were found in metabolic rate, RER, rate of fat oxidation or carbohydrate oxidation measured 2 hours after the administration of a 75 g glucose load (Table 3.4). The magnitude of the change (value at 2 hours after glucose load minus fasting value) in metabolic rate, RER, fat and carbohydrate oxidation rates was not significant different between the groups (data not shown).

Table 3.4 Metabolic rate, respiratory exchange ratio (RER), rates of fat and carbohydrate (CHO) oxidation in Controls and Offspring in the fasted state and 2 hours after glucose load.

	Controls (n=36)	Offspring (n=34)	P - value
Resting metabolic rate (kJ.kg ⁻¹ .min ⁻¹)	0.06 ± 0.01	0.06 ± 0.01	0.43
Post-glucose metabolic rate (kJ.kg ⁻¹ .min ⁻¹)	0.06 ± 0.007	0.06 ± 0.008	0.29
Fasting RER	0.86 ± 0.05	0.85 ± 0.05	0.66
Post-glucose RER	0.95 ± 0.04	0.95 ± 0.05	0.67
Fasting fat oxidation (mg.kg ⁻¹ .min ⁻¹)	0.47 ± 0.33	0.50 ± 0.26	0.68
Post-glucose fat oxidation (mg.kg ⁻¹ .min ⁻¹)	0.07 ± 0.18	0.04 ± 0.23	0.56
Fasting CHO oxidation (mg.kg ⁻¹ .min ⁻¹)	1.85 ± 0.91	1.69 ± 0.68	0.42
Post-glucose CHO oxidation (mg.kg ⁻¹ .min ⁻¹)	3.01 ± 0.69	2.95 ± 0.80	0.72

Values are mean ± SD. P-values are for unpaired *t*-tests between groups.

3.3.5 Blood pressure measurement and peripheral pulse wave velocity

No significant differences in fasting systolic or diastolic blood pressure were observed (Table 3.5). In addition, no differences in pulse wave velocity were observed between Controls and Offspring in either the fasted or post-glucose state (Table 3.5).

Table 3.5 Fasting systolic and diastolic blood pressure and peripheral pulse wave velocity (PWV) in fasting and at 2 hours after glucose load.

	Controls (n=36)	Offspring (n=34)	P - value
Systolic blood pressure (mmHg)	117.4 ± 12.7	120.3 ± 14.3	0.37
Diastolic blood pressure (mmHg)	72.1 ± 8.9	76.4 ± 11.1	0.07
Fasting PWV (m.s ⁻¹) ^a	9.5 ± 1.5	9.7 ± 1.6	0.79
Post-glucose PWV (m.s ⁻¹) ^a	9.4 ± 1.3	9.6 ± 1.6	0.57

Values are mean ± SD. P-values are for unpaired *t*-tests between groups. ^aStatistical analysis performed on logarithmically transformed data.

3.3.6 Plasma lipids

No significant differences were found in total cholesterol, LDL-cholesterol, HDL cholesterol or triglycerides between Offspring and Controls (Table 3.6).

Table 3.6 Fasting concentrations of total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol in Offspring and Control groups.

	Controls	Offspring	p-value
	(n=36)	(n=34)	
Total cholesterol (mmol.l ⁻¹)	4.23 ± 1.04	4.17 ± 1.06	0.80
Fasting triglycerides (mmol.l ⁻¹) ^a	0.86 ± 0.30	0.98 ± 0.36	0.14
HDL cholesterol (mmol.l ⁻¹)	1.22 ± 0.31	1.27 ± 0.28	0.55
LDL cholesterol (mmol.l ⁻¹)	2.62 ± 0.94	2.45 ± 0.94	0.48

Values are mean ± SD. P-values are for unpaired *t*-tests between groups. ^astatistical analysis performed on logarithmically transformed data.

3.3.7 Liver enzymes

No significant differences in fasting values for GGT (Control, 13.6 ± 8.6 mmol.l⁻¹, Offspring 20.1 ± 21.4 mmol.l⁻¹, *p* = 0.35) or ALT (Control 16.9 ± 6.5 mmol.l⁻¹, Offspring 17.5 ± 7.6 mmol.l⁻¹, *p* = 0.92) were found between Controls and Offspring.

3.3.8 Adipokines & C-reactive protein

Fasting plasma concentrations of adiponectin, leptin, TNFα, IL-6, resistin and CRP are presented in Table 3.7 and Figure 3.4. No significant differences were found between Controls and Offspring for any of the adipokines measured but there was a trend towards higher resistin concentrations in the Offspring group. No significant difference was observed for CRP concentration between groups.

Table 3.7 Fasting plasma adipokine concentrations in Control and Offspring groups.

	Controls (n=36)	Offspring (n=34)	P - value
Adiponectin ($\mu\text{g.ml}^{-1}$) ^a	7.4 \pm 2.8	7.2 \pm 3.2	0.66
Leptin (ng.ml^{-1}) ^a	17.8 \pm 10.8	22.2 \pm 14.7	0.33
TNF- α (pg.ml^{-1}) ^a	1.3 \pm 0.8	1.1 \pm 0.5	0.47
IL-6 (pg.ml^{-1}) ^a	1.7 \pm 1.4	1.4 \pm 0.9	0.58
Resistin (ng.ml^{-1})	14.5 \pm 4.2	16.9 \pm 5.8	0.06
CRP (mmol.l^{-1}) ^a	2.5 \pm 3.0	2.3 \pm 3.4	0.74

Values are mean \pm SD. P-values are for unpaired *t*-tests between groups. ^astatistical analysis performed on logarithmically transformed data.

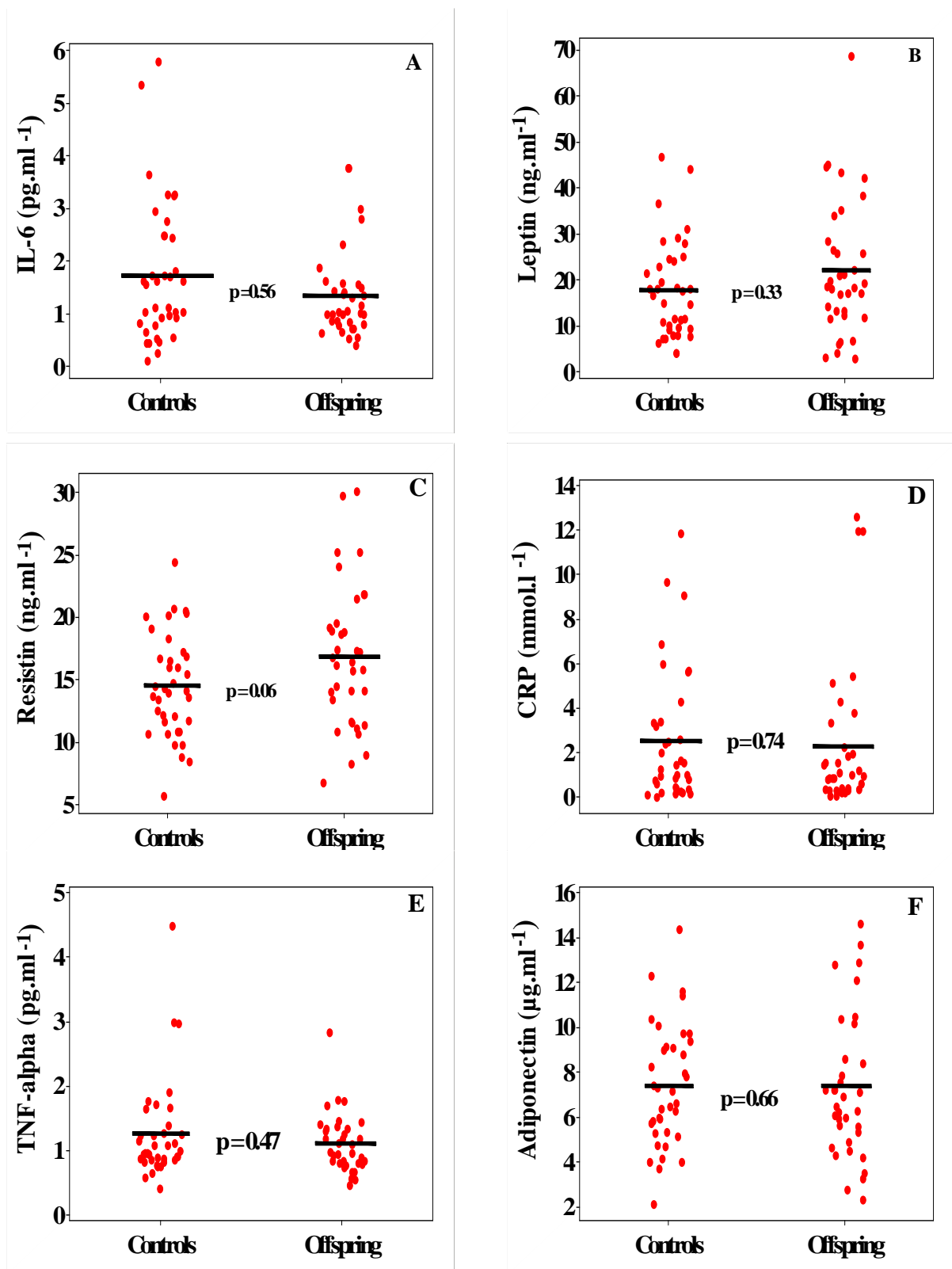


Figure 3.4 Individual value plots of baseline adipokines in Controls and Offspring. p-values are for unpaired t-tests between groups. Statistical analysis performed on logarithmically transformed data for all adipokines except resistin.

In both Control and Offspring groups, fasting adiponectin concentrations correlated positively with ISI (Table 3.8). After adjustment for fat mass, Offspring alone continued to display a significant positive association between fasting adiponectin concentration and ISI (Table 3.8, Figure 3.5). Leptin concentration was negatively correlated with ISI in both groups, but was no longer statistically significant in either group after adjustment for fat mass (Table 3.8). IL-6 concentration was negatively correlated with ISI in Offspring alone, but this observation was no longer statistically significant after adjustment for fat mass (Table 3.8). CRP was negatively correlated with ISI in both Controls and Offspring, but remained significant in Offspring alone after adjustment for fat mass (Table 3.8, Figure 3.6).

Table 3.8 Correlations between adiponectin, leptin, TNF- α , IL-6, resistin and CRP concentrations and ISI in Controls (n = 36) and Offspring (n = 34), before and after adjustment for fat mass.

		Controls		Offspring	
		Unadjusted	Adjusted	Unadjusted	Adjusted
Adiponectin	r	0.38*	0.07	0.51**	0.56***
Leptin	r	-0.57***	-0.17	-0.52**	-0.05
TNF- α	r	0.16	0.19	-0.22	-0.20
IL-6	r	-0.22	0.06	-0.37*	-0.07
Resistin	r	-0.20	0.18	-0.12	-0.15
CRP	r	-0.36*	0.05	-0.66***	-0.41*

r = Univariate correlations of variable vs. ISI. Unadjusted = variable correlated against ISI.

Adjusted = variable correlated against residual of ISI and fat mass. ^astatistical analysis

performed on logarithmically transformed data. Statistically significant correlation shown in

bold. * p < 0.05, ** p < 0.01, *** p < 0.001.

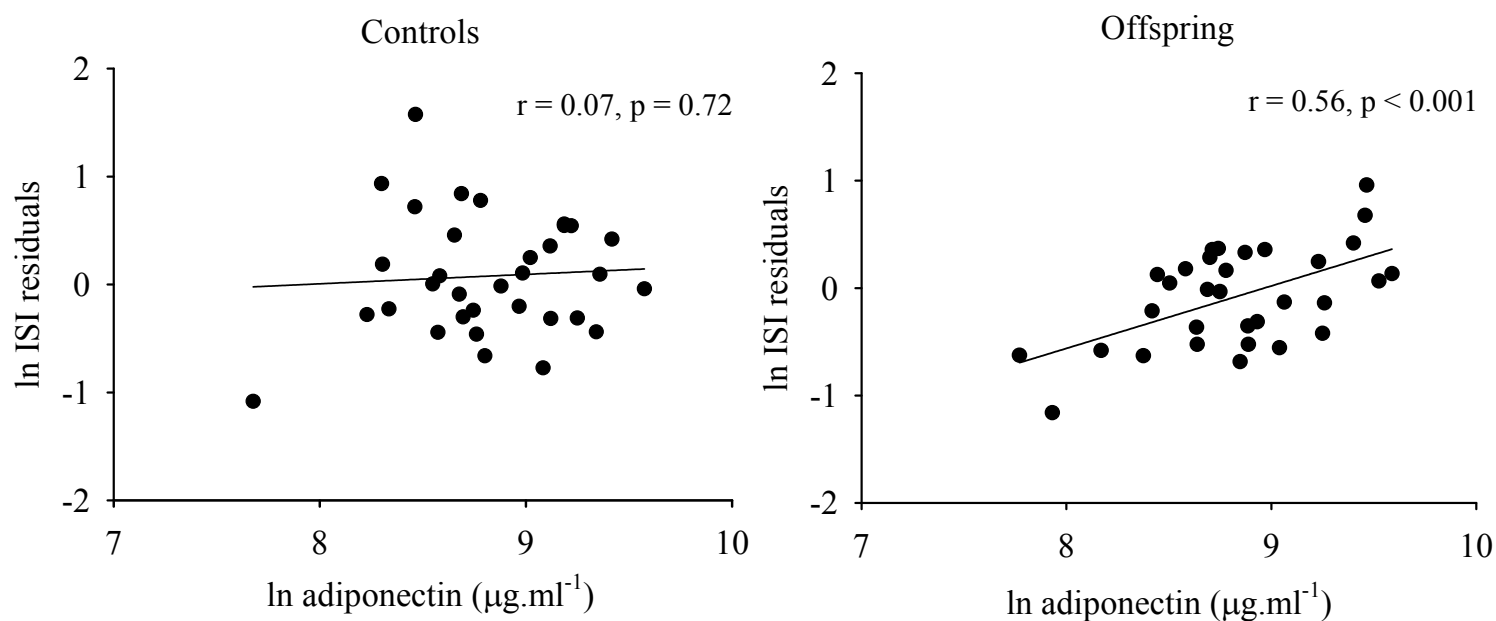


Figure 3.5 Scattergram showing correlation between ln adiponectin and ln ISI residual after adjustment for fat mass in Controls and Offspring.

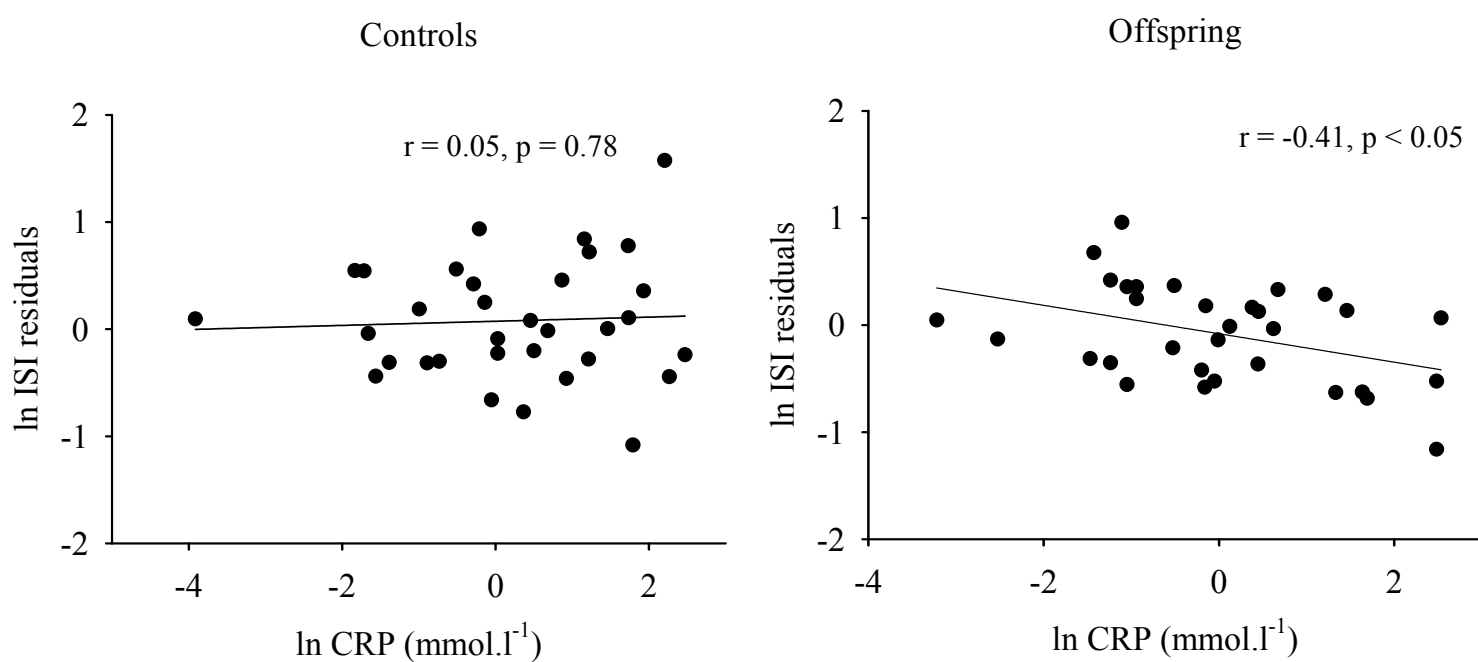


Figure 3.6 Scattergram showing correlation between ln CRP and ln ISI residual after adjustment for fat mass in Controls and Offspring.

3.3.9 Cardiorespiratory Fitness & Habitual Physical Activity

There was no significant difference between $\dot{V}O_{2\max}$ in Controls compared to Offspring (Table 3.9). Lactate threshold occurred at a similar absolute $\dot{V}O_2$ in both Controls and Offspring (Table 3.9). There were no observed differences in resting heart rate or average daily heart rate. Controls displayed a trend towards a higher average heart rate minus resting heart rate (a surrogate measure of total activity) (Table 3.9). In addition, Controls spent significantly greater amounts of time per day at heart rates which were twice that of resting ($p < 0.05$) (Table 3.9).

Table 3.9 Indices of cardiorespiratory fitness and physical activity in Control and Offspring groups.

	Controls (n=36)	Offspring (n=34)	P - value
$\dot{V}O_{2\max}^b$ (ml.kg ⁻¹ .min ⁻¹)	32.7 ± 6.7	30.4 ± 4.8	0.10
$\dot{V}O_2$ at lactate threshold (ml.kg ⁻¹ .min ⁻¹) ^{b,a}	21.0 ± 4.1	21.0 ± 3.8	0.97
Resting HR ^c (b.min ⁻¹)	66.1 ± 10.0	69.6 ± 7.8	0.15
Average daily HR (b.min ⁻¹)	85.7 ± 8.6	86.0 ± 6.3	0.85
Average daily HR - resting HR	19.6 ± 1.11	16.5 ± 1.10	0.05
Time spent above 1.5 times resting HR (min.day ⁻¹) ^a	133.0 ± 130.9	86.7 ± 65.7	0.15
Time spent above 2 times resting HR (min.day ⁻¹)	18.4 ± 28.8	6.3 ± 6.3	< 0.05

Values are mean ± SD. P-values are for unpaired *t*-tests between groups. ^astatistical analysis performed on logarithmically transformed data. ^bEstimated from submaximal incremental treadmill test. ^cFrom pulse wave velocity determination. $\dot{V}O_2$: Oxygen uptake; $\dot{V}O_{2\max}$: Maximal oxygen uptake; HR: heart rate.

The Control group displayed significant negative associations between resting heart rate, average daily heart rate and ISI. After adjustment for fat mass, only resting heart rate remained significantly negatively correlated with ISI (Table 3.10). Time spent at 1.5 times resting heart rate was significantly positively associated with ISI in the Control group, and this association remained significant after adjustment for fat mass (Table 3.10). In the Offspring group, ISI correlated negatively with resting heart rate and positively with time spent at 1.5 times resting heart rate but these associations became non significant after adjustment for fat mass (Table 3.10). Controls exhibited a positive correlation between predicted $\dot{V}O_{2max}$ and ISI, and $\dot{V}O_2$ at lactate threshold and ISI (Table 3.10). However, these significant associations were lost after adjustment for fat mass (Table 3.10). The Offspring group did not display any significant associations between indices of cardiorespiratory fitness and ISI (Table 3.10).

Table 3.10 Correlations between indices of physical activity and ISI, and cardiorespiratory fitness and ISI in Controls and Offspring, before and after adjustment for fat mass.

		Controls		Offspring	
		Unadjusted	Adjusted	Unadjusted	Adjusted
$\dot{V}O_{2\max}$ (ml.kg ⁻¹ .min ⁻¹)	r	0.40*	0.06	0.20	-0.15
$\dot{V}O_2$ at lactate threshold (ml.kg ⁻¹ .min ⁻¹) ^a	r	0.46**	0.11	0.29	-0.04
Resting HR	r	-0.59***	-0.46*	-0.47*	-0.37
Average daily HR	r	-0.50**	-0.30	-0.32	-0.23
Average daily HR - resting HR	r	0.27	0.33	0.29	0.25
Time spent above 1.5 times resting HR ^a	r	0.53**	0.46*	0.39*	0.27
Time spent above 2 times resting HR	r	0.33	0.23	0.23	0.08

r = Univariate correlations of variable vs. ISI, Adjusted R = correlation of variable vs. ISI adjusted for influence of fat mass. ^astatistical analysis performed on logarithmically transformed data. Statistically significant correlation shown in bold.* p < 0.05, ** p < 0.01, ***p < 0.001.

3.3.10 Energy intake and dietary composition

Data on energy and macronutrient intake are presented in Table 3.11. No significant differences were observed in energy or macronutrient intake between Control and Offspring groups (Table 3.11). No dietary factors were associated with ISI in the Control or Offspring groups.

Table 3.11 Energy and macronutrient intake in Control and Offspring groups.

	Controls (n=32)	Offspring (n=32)	P - value
Energy (kJ.kg ⁻¹ .day ⁻¹)	108 ± 27	114 ± 34	0.39
Total fat (g. kg ⁻¹ .day ⁻¹)	0.90 ± 0.27	1.00 ± 0.32	0.17
SFA (g. kg ⁻¹ .day ⁻¹) ^a	0.32 ± 0.12	0.35 ± 0.12	0.19
MUFA (g. kg ⁻¹ .day ⁻¹)	0.29 ± 0.09	0.34 ± 0.12	0.10
PUFA (g. kg ⁻¹ .day ⁻¹) ^a	0.13 ± 0.06	0.20 ± 0.10	0.15
Total CHO (g. kg ⁻¹ .day ⁻¹) ^a	3.36 ± 1.04	3.57 ± 1.41	0.68
Sugar (g. kg ⁻¹ .day ⁻¹) ^a	1.52 ± 0.78	1.62 ± 0.86	0.66
Starch (g. kg ⁻¹ .day ⁻¹) ^a	2.50 ± 4.01	1.95 ± 0.72	0.76
Protein (g. kg ⁻¹ .day ⁻¹) ^a	1.00 ± 0.25	1.00 ± 0.28	0.62

Values are mean ± SD. P-values are for unpaired *t*-tests between groups. ^astatistical analysis performed on logarithmically transformed data. SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids, CHO: Carbohydrate.

3.4 Discussion

As expected (Higgins et al. 2005;Perseghin et al. 1997;Humphriss et al. 1997;Straczkowski et al. 2003;Nyholm et al. 2004;Ahn et al. 2004) it was observed that sedentary female offspring of type 2 diabetic patients were more insulin resistant than matched females without a family history of type 2 diabetes and that adiposity is an important contributing factor to insulin sensitivity in both Offspring and Control groups. Most importantly, by using accurate and detailed measurements of body composition it was demonstrated that the effect of adiposity on insulin sensitivity is influenced by a family history of diabetes. Indeed, the Offspring group was found to have stronger and more consistent negative associations between adiposity indices and ISI than Controls. In contrast to reports from others (Higgins et al. 2005;Adamson et al. 2001), there were no differences in dietary intake between Controls and Offspring and insulin sensitivity was not related to diet in either group. Consistent with previous evidence, Offspring were less habitually active but contrary to previous evidence from Higgins and colleagues (2005), Controls showed a stronger association than Offspring between physical activity and insulin sensitivity (Higgins et al. 2005).

Although there is some evidence that in offspring of type 2 diabetic patients, measures of adiposity (Humphriss et al. 1997;Perseghin et al. 1997;Nyholm et al. 2004;Higgins et al. 2005) or body mass index (Adamson et al. 2001;Higgins et al. 2005) are increased in comparison with control subjects, in this study no differences in body mass, body mass index or measurements of adiposity were observed between Offspring and Control groups.

Although measurements of adiposity such as BMI, total body fat, regional body fat, skinfold measurements and waist and hip circumferences were not different between the groups, the associations between adiposity markers and insulin sensitivity were stronger in the Offspring group. Cross-sectional analysis of heterogenous groups of relatives and controls has previously suggested greater susceptibility to the effect of adiposity on insulin sensitivity in relatives (van Dam et al. 2001). However, subsequent studies have not provided evidence of

this in matched groups of relatives and controls (Nyholm et al. 2004;Johanson et al. 2003;Higgins et al. 2005). The results of the current study are important since contrary to the previous research, it would appear that insulin sensitivity is more susceptible to the influence of adipose tissue in Offspring than in Controls.

Other groups who are predisposed to the development of type 2 diabetes (e.g. South Asians, Pima Indians) are recognised to display insulin resistance at lower levels of adiposity than matched subjects who are thought to have a lower predisposition for the condition (Gautier et al. 1999;Razak et al. 2007). South Asians also manifest both insulin resistance and diabetes at lower BMI values than Caucasians (Razak et al. 2007). Indeed, comparison between BMI matched South Asian and Caucasian males revealed similar visceral fat mass, but larger subcutaneous adipose mass in South Asians (Chandalia et al. 2007). Sniderman and colleagues (2007) suggest that this group has elevated deep subcutaneous adipose mass, which has greater contribution to insulin resistance (Sniderman et al. 2007). Thus, the current study findings support the notion that relatives, similar to other high-risk groups, have greater susceptibility to the adverse effects of obesity on glycaemia and insulin sensitivity than their counterparts with no family history of diabetes (Johanson et al. 2003;van Dam et al. 2001).

Although no differences were observed in cardiorespiratory fitness between the two groups, Offspring displayed a trend towards a lower total activity, calculated as average daily heart rate minus resting heart rate, and spent significantly less time per day at heart rates which were two times higher than resting heart rates. Thus, in this study Offspring were significantly less active than Control subjects. This is consistent with the work of Higgins and colleagues (2005) which reported that offspring display lower daily energy expenditure estimated from 7 day activity diaries (Higgins et al. 2005). However, correlations between physical activity measures and ISI found in the current study were different from those reported by Higgins and colleagues (2005). In the current study, only Controls displayed

significant positive correlations between indices of physical activity (such as time spent at 1.5 times resting heart rate) and ISI, after adjustment for fat mass, whilst Higgins and co-workers observed that HOMA_{IR} was significantly negatively correlated with habitual levels of daily activity only in non-diabetic daughters of patients with type 2 diabetes (Higgins et al. 2005). Therefore, the notion that in Offspring, insulin sensitivity is influenced by physical activity to a greater degree than in individuals without family history of diabetes was not confirmed in this study.

Reasons for these differences may be related to the differing methods used to evaluate physical activity. The heart rate analysis utilised in this study is a more accurate and objective description of physical activity than the estimated energy expenditure method used by Higgins and colleagues (Higgins et al. 2005). In addition, the subject group in the current study were different to those described by Higgins and co-workers. The current study specified that all volunteers were sedentary, and results suggest that Offspring in the current study were more sedentary than the Control groups. The absence of significant correlations between physical activity and ISI in Offspring might suggest that habitual physical activity does not play a major role in the determination of insulin sensitivity. However, it should be appreciated that the range of physical activity in the Offspring volunteers showed less variability than Controls, and this homogeneity of physical activity may preclude detection of a direct interaction. Additionally, despite the absence of a direct relationship between physical activity and ISI it should be appreciated that physical activity may have an indirect effect through other mechanisms. One such mechanism is a potential alteration in partitioning of dietary saturated fats, which in the context of extreme physical inactivity, promotes palmitate storage in muscle and contributes to increased intramuscular lipid content (Bergouignan et al. 2009).

In agreement with previous studies (Johanson et al. 2003; Higgins et al. 2005), there were no significant differences in daily energy and macronutrient intake or proportion of energy provided by the main energy providing nutrients between Offspring and Control groups. Furthermore, in both Control and Offspring groups dietary factors did not correlate with insulin sensitivity. Taken together these data suggest that impaired insulin sensitivity seen in Offspring can not be explained by the difference in dietary factors. It should be noted however that other studies (Adamson et al. 2001) found that non-diabetic relatives of type 2 diabetic patients consumed diets which are expected to promote the development of diabetes (Adamson et al. 2001). Indeed, in the above study (Adamson et al. 2001) relatives were reported to have higher absolute intakes of total fat, saturated fat and cholesterol and lower intake of non-starch polysaccharides and suggested that there is scope for decreasing the disease risk through improvements in the diet (Adamson et al. 2001). However, relatives studied by Adamson and colleagues (2001) were significantly heavier, with higher waist circumferences than controls and may therefore be considered to be of a different metabolic phenotype than those described in this study (Adamson et al. 2001). Indeed, consideration of dietary habits may be of a particular importance since the effects of dietary fatty acids may differ between offspring of type 2 diabetic patients and individuals without family history of diabetes. Negative associations between saturated fat and insulin sensitivity, and a positive association between unsaturated fats and insulin sensitivity have been observed in relatives alone (Ntali et al. 2009).

This study also aimed to find whether early metabolic abnormalities seen in Offspring can be explained by impairment in metabolic flexibility. Metabolic inflexibility has been defined as an increase in muscle glucose oxidation in fasting conditions, and decreased glucose oxidation under insulin-stimulated circumstances (Kelley & Mandarino 2000) and has been shown to contribute to the accumulation of intramuscular triglyceride and thus insulin resistance (Felber et al. 1987; Boden 1997; Delarue & Magnan 2007). In this study however, there were no differences in the rate of fat and carbohydrate oxidation in fasted or post-

glucose states between the Offspring and Control groups. To some extent this finding is not surprising, since precise investigation of metabolic inflexibility has been performed most successfully in isolated tissue groups (Kelley & Mandarino 2000) and there is evidence that ‘whole body’ respiratory quotient may be a poor index of a metabolic abnormality which is predominantly skeletal-muscle based (Galgani et al. 2008; Galgani, Moro, & Ravussin 2008). In addition, metabolic inflexibility is typically observed in obese and insulin resistant subjects who display evidence of elevated circulating NEFA concentrations (Delarue & Magnan 2007; Corpeleijn, Saris & Blaak 2009). The Offspring group in this study were observed to have neither greater adiposity, nor higher NEFA concentrations.

Although NEFA concentration was not different between the two groups, this is not surprising, since there were no obvious differences in subcutaneous abdominal adipose tissue mass which is the major determinant of systemic NEFA concentrations (Bickerton et al. 2008; Frayn et al. 1993). Additionally, Bickerton and colleagues (2008) have demonstrated relatively suppressed NEFA concentrations due to hyperinsulinaemia in insulin resistant subjects (Bickerton et al. 2008). Therefore, in the current Offspring group a tendency towards increased circulating NEFA may have been suppressed by hyperinsulinaemia. After adjustment for fat mass, NEFA was significantly negatively correlated with ISI in Offspring alone, suggesting that women with a family history of type 2 diabetes may be more sensitive to the effects of fatty acids on insulin sensitivity (Roden et al. 2000; Boden 1997; Boden & Shulman 2002).

Single measurements of NEFA concentrations over an OGTT may be misleading in terms of the metabolic interaction between lipolysis in adipose tissue and NEFA uptake in skeletal muscle. *In vivo* NEFA flux between adipose tissue and skeletal muscle could be greater in Offspring than in Control volunteers and may represent insulin resistance in adipose tissue and greater NEFA uptake by insulin resistant skeletal muscle. Confirmation of this

supposition would require a study of NEFA efflux from adipose tissue under OGTT conditions. However this would have required superficial abdominal vein cannulation (Frayn, Coppack, & Humphreys 1993) and forearm cannulation to determine arteriovenous differences (Evans, Clark, & Frayn 1999). This would have provided information about lipolysis in adipose tissue in response to an oral glucose load and subsequent increase in circulating insulin concentration and the skeletal muscle uptake of increased NEFA efflux. Measurement of the VLDL-triglyceride arteriovenous difference in skeletal muscle would have given evidence of increased local delivery of NEFA from circulating VLDL-triglyceride, since skeletal muscle LPL activity is dependent on plasma lipid concentrations (Frayn, Arner, & Yki-Jarvinen 2006). In this study fasting triglyceride concentration was not different between Controls and Offspring but no assessment was made of plasma triglyceride concentration over the OGTT. Future exploration of the early mediators of insulin resistance in Offspring could include both analysis of lipid kinetics in both adipose tissue and skeletal muscle and lower doses of oral glucose in order to assess more subtle changes in glucose-insulin-lipid dynamics (Bickerton et al. 2007).

No differences were observed in concentrations of adipokines, however in Offspring alone, adiponectin was significantly positively correlated with ISI, independent of fat mass. In Offspring alone, these findings may indicate an important role for adipose tissue function, independent of total adipose mass, in determining ISI. In particular, Offspring appear to be more sensitive to the influences of these factors on insulin sensitivity. Fasting adiponectin is an independent predictor of both liver fat and insulin sensitivity and in addition to promotion of both fatty acid uptake and oxidation in muscle cells, is thought to increase hepatic insulin sensitivity through stimulation of peroxisome proliferators-activated receptor alpha (PPAR α) (Furler et al. 2006; Yamauchi & Kadowaki 2008; Koska et al. 2008). Similar to the potential relationship between NEFA and ISI observed in this study, the positive correlation between ISI and adiponectin (independent of fat mass) in Offspring may reflect a compensatory response to the effects of NEFA on insulin sensitivity in muscle and liver, perhaps reflecting

subjects who are able to counteract increased NEFA by adiponectin mediated stimulation of hepatic fatty acid oxidation (Bonet et al. 2007). In this study, supportive evidence for this suggestion may be considered by the absence of elevated biomarkers of hepatic steatosis (ALT, GGT) (Fraser et al. 2009) in Offspring, which is consistent with previously observed normal hepatic lipid content in male offspring subjects despite the presence of insulin resistance (Johanson et al. 2003).

The significance of CRP in the pathogenesis of insulin resistance remains controversial. Elevated concentrations of CRP are associated with both cardiovascular disease and insulin resistant states (Festa et al. 2000; Pradhan et al. 2003; Tesaro et al. 2007). However, it is possible that CRP is simply a marker of the chronic inflammatory state common to both cardiovascular disease and insulin resistance, and as yet, no causal link has been identified (Sattar & Lowe 2006; Kriketos et al. 2004). The current study reports a negative correlation between CRP and ISI in Offspring alone. This is thought to indicate a low grade chronic inflammatory state which is implicated in the pathogenesis of insulin resistance and vascular dysfunction, and appears to be associated with increased visceral fat mass (Kowalska et al. 2008; Salmenniemi et al. 2005). The current study did not determine corroborative evidence of a pro-inflammatory state, increased visceral fat mass or altered vascular function in Offspring.

This study has advantages over previous studies using objective assessment of body composition by using DEXA scanning. In addition, objective measurements of both cardiorespiratory fitness and habitual physical activity were performed, allowing a more complete description of both physical fitness and daily activity. Furthermore, use of accurate values of total fat mass allowed correction for this factor and an understanding of the influences upon insulin sensitivity which cannot be directly attributed to fat mass.

There are areas which would strengthen this study further. Direct imaging using computed tomography or magnetic resonance imaging would provide a direct assessment of visceral fat and partitioned subcutaneous fat, which would be of great value when further considering the influences upon hepatic insulin sensitivity and adjusting for the influence of fat mass. Dietary data is likely to have been under-reported, however more robust evaluation of dietary intake is difficult in free-living subjects (Hill & Davies 2001; Weber et al. 2001). This study was unable to assess habitual energy expenditure, due to the limitations in the application of the FLEX technique. It would also be reasonable to consider the use of venous occlusion plethysmography as a tool for vascular assessment since it may be a more useful tool in assessing skeletal muscle microvascular function, although more invasive methods such as contrast enhanced ultrasound would be more robust (Clark 2008; Wilkinson & Webb 2001). The gold standard technique, the euglycemic hyperinsulinaemic clamp was not used for the assessment of insulin sensitivity. However, the insulin sensitivity index that was used has been shown to correlate well with the rate of whole-body glucose disposal during a euglycemic hyperinsulinaemic clamp ($r = 0.73$, $p < 0.0001$), and can be considered to be a more physiological index of insulin sensitivity than $HOMA_{IR}$ which is primarily an index of hepatic insulin sensitivity (Matsuda & DeFronzo 1999; Chang et al. 2006). Use of the OGTT was a more practical method of assessing insulin sensitivity and performing 70 clamp studies was not feasible in the available timescale.

In conclusion, this study observed differences in the metabolic phenotype of Offspring characterised by lower insulin sensitivity. In addition, adiposity more strongly influenced insulin sensitivity in Offspring, and circulating fatty acids were also associated with insulin sensitivity in Offspring alone, regardless of fat mass. Adiponectin was positively correlated with ISI in Offspring alone, and CRP negatively correlated in this group only. These differences were apparent despite similar dietary intake, but were associated with lower

habitual activity levels in Offspring, whose insulin sensitivity was not influenced by physical activity. These observations suggest an important role for adipose tissue and lipotoxicity in the early pathogenesis of insulin resistance, with possible early compensatory mechanisms, in sedentary premenopausal daughters of patients with type 2 diabetes.

CHAPTER 4

PHYSICAL AND METABOLIC RESPONSES TO A STRUCTURED EXERCISE PROGRAMME IN WOMEN WITH AND WITHOUT A FAMILY HISTORY OF TYPE 2 DIABETES

4.1 Introduction

The rapid increase in the prevalence of type 2 diabetes over recent years (Wild et al. 2004) must be attributed to changes in environmental factors, such as body fatness and exercise habits, as the human genome has not changed over this short period (Booth et al. 2000). Epidemiological data suggests that rates of both overweight/obesity and increasing urbanisation are closely related to prevalence of diabetes (King, Aubert, & Herman 1998). Prospective observational data utilising proportional hazard models (Colditz et al. 1995) and multiple logistic regression modelling (Manson et al. 1992) suggests that being overweight with an abdominal fat distribution probably accounts for 80-90% (Astrup & Finer 2000), and that a sedentary lifestyle accounts for at least 25% (Manson et al. 1992), of all type 2 diabetes incidence. However, as not all overweight and sedentary individuals develop the disease, it is clear that other non-environmental factors also play an important role in determining risk for type 2 diabetes. In sedentary populations, first-degree relatives of patients with type 2 diabetes have about three times the risk of developing diabetes than their counterparts with no family history of diabetes (Kobberling & Tillil 1982; Ohlson et al. 1988) and even when matched for BMI, are often more insulin resistant than control participants (Kriketos et al. 2004; Nyholm et al 2004; Perseghin et al 1997), suggesting that relatives have an innate susceptibility (probably due to some combination of genetic, epigenetic and/or 'early-origins' factors) to insulin resistance and diabetes. However, in epidemiological observations, the excess risk of developing diabetes that is associated with a diabetes family history is much greater in sedentary population groups than in physically active population groups, suggesting that the increased risk associated with a diabetes family history may be diminished (although not eliminated) at high levels of physical activity (Hu et al.

1999;Sargeant et al. 2000). In addition, recent cross-sectional reports suggest that modulation of insulin resistance in offspring of patients with type 2 diabetes may be particularly amenable to habitual physical activity or cardiorespiratory fitness level, such that inactive or unfit offspring are insulin-resistant compared with control participants with no diabetes family history, whereas active or fit offspring exhibit levels of insulin sensitivity that are much closer to persons with no diabetes family history (Ahn et al. 2004;Gill & Malkova 2006;Higgins et al. 2005). Taken together, these observations suggest that healthy offspring of patients with type 2 diabetes possess an innate predisposition to insulin resistance, with consequent increased diabetes risk, which manifests when sedentary. However, this appears to be highly modulated by level of physical activity. It is therefore hypothesised that, in response to the same exercise training intervention, offspring of type 2 diabetes patients would improve insulin sensitivity to a greater extent than control participants with no diabetes family history.

4.2 Methods

4.2.1 Volunteers

Volunteers were recruited and screened as detailed in section 2.1. Specific inclusion criteria are defined in section 2.1.3. Further information detailing recruitment response and excluded volunteers can be found in Figure 2.2. The Offspring group was composed of women with a first-degree relative with type 2 diabetes (T2D). The Control group was composed of women with no first or second degree relative with T2D. This study was conducted on twenty eight Offspring and thirty four Control volunteers. Control and Offspring groups were well matched for age (Controls 33.5 ± 6.3 years, Offspring 35.5 ± 6.7 years, $p = 0.22$) and BMI (Controls 27.0 ± 4.3 kg.m⁻², Offspring 28.1 ± 4.9 kg.m⁻², $p = 0.36$).

4.2.2 Study Design

Volunteers were randomised into 'Exercise' or 'Delayed-Exercise' intervention arms using a computer programme. The study design is shown in Figures 2.3-2.4. The testing regime for each intervention arm is described in Section 2.1.3. All post-intervention OGTTs were performed 15-24 hours after the volunteers' final exercise session. The Exercise group participated in further metabolic testing 3 days after the final exercise session (Figure 2.4), during which period the volunteers were asked to avoid any planned exercise, to assess the acute and chronic effects of the exercise intervention. Analysis of the exercise-mediated change in variables was made by comparison of baseline and acute post-intervention data in the Exercise group, and by comparison of the data collected after the period of 'usual lifestyle' (prior to inclusion in the exercise intervention) and the post-intervention data in the Delayed-Exercise group. Therefore irrespective of the intervention arm, volunteers were studied at eight-week intervals to allow, as far as possible, for all metabolic, fitness and body composition assessments for each individual to be made in the same menstrual cycle phase

(Creinin, Keverline, & Meyn 2004). Volunteers were asked to maintain their usual dietary and habitual physical activity habits throughout the studied periods.

4.2.3 Fitness testing

Cardiorespiratory fitness was measured by predicted $\dot{V}O_{2\max}$ and lactate threshold, as described in section 2.6.

4.2.4 Metabolic testing

Volunteers attended for metabolic testing as described in section 3.2.8. Assessment of metabolic rate and substrate utilisation was performed in the fasted state and 2 hours after a 75 g oral glucose load (section 2.3.2). Additionally, pulse wave velocity and blood pressure were measured in the fasted and post-glucose state as described in section 2.3.4 – 2.3.5. Fasting plasma samples were analysed as described in sections 2.4.2 and 2.4.3. An OGTT was performed using a 75 g oral glucose load with sampling from an intravenous cannula sited in the antecubital fossa, as described in section 2.3.7. Samples were analysed at 30, 60, 90 and 120 minutes for insulin, glucose, NEFA and 3-OHB (Section 2.4).

4.2.5 Body composition assessment

Dual X-Ray Absorptiometry (DEXA) scans (LUNAR Prodigy DEXA scanner, GE Healthcare Diagnostic Imaging, Slough, Berkshire, UK) were used to determine body composition and fat distribution (section 2.2.5). Height, body mass, waist and hip circumferences, and biceps, triceps, suprailiac and subscapular skinfolds were also determined using standard protocols (section 2.2) (Marfell-Jones M et al. 2006).

4.2.6 Assessment of diet and habitual physical activity

Self-reported dietary intake and activity diaries were completed for a seven-day period prior to each episode of metabolic, anthropometric and fitness testing (Section 2.8-2.9). Seven day waking-hours heart rate monitoring was also performed prior to each testing episode (Section 2.9). Indices of habitual physical activity were calculated as detailed in section 2.10.

4.2.7 Exercise intervention

Volunteers participated in a 7-week endurance-type exercise training programme with weekly supervised sessions as described in section 2.7.

4.3 Statistical analysis

Data were analysed using Statistica (version 6.0, StatSoft Inc., Tulsa, Oklahoma) and Minitab (version 13.1, Minitab Inc., State College, Pennsylvania). Prior to analysis, all data were tested for normality using the Anderson-Darling normality test and, if necessary, logarithmically transformed. Based on data from the two baseline measurements made in the volunteers randomised to the Delayed-exercise intervention arm, the within-subject test-retest coefficient of variation for ISI was 12.2%, with mean values for ISI differing by 3.2% between the two baseline measurements. Changes from the immediately pre-intervention assessment to the post-intervention assessment in all volunteers who completed the intervention (i.e. between the first and second sets of tests in volunteers randomised to the 'Exercise' intervention and between the second and third sets of tests in volunteers randomised to 'Delayed-Exercise' intervention) were compared by 2-way ANOVA (group x trial) with repeated measures on the 'trial' factor. The group x trial interaction term was used to determine whether the Offspring and Control subjects responded differently to the intervention and *post-hoc* Tukey tests were used to identify in which group(s) changes occurred. In separate analyses, similar comparisons were made between the first and second

sets of tests in volunteers randomised to the 'Delayed-Exercise' intervention, to determine whether a 'study effect' could have contributed to the observed changes. Additionally, comparisons were made between the second and third sets of tests in volunteers randomised to the 'Exercise' intervention, to determine whether a three day period without planned exercise would result in further metabolic changes. Associations between variables were assessed using univariate linear correlations, with adjustments for the influence of change in other variables as described in Section 3.2.9.2. Multiple regression analyses were performed to establish the independence of relationships.

4.3.1 Calculation of glucose, insulin, NEFA, and 3-OHB concentrations and insulin sensitivity during oral glucose tolerance test

Concentrations of glucose, insulin, NEFA and 3-OHB were measured as described in section 2.4. Mean concentrations of glucose, insulin, NEFA and 3-OHB during the OGTT were calculated as described in section 3.2.9.1. Insulin sensitivity was expressed as the Insulin Sensitivity Index (Matsuda & DeFronzo 1999) and calculated as described in section 2.11.2.

4.4 Results

4.4.1 Changes during the usual lifestyle period for the Delayed Exercise group

There were no significant changes in energy intake or dietary composition, or in indices of habitual physical activity over the course of the 7-week 'usual lifestyle' period in either the Control (n=16) or the Offspring (n=13) (data not shown). The only significant changes in metabolic variables during the usual lifestyle period were observed in the Offspring group. Offspring showed an increase in fasting triglycerides (Pre: $0.91 \pm 0.07 \text{ mmol.l}^{-1}$; Post $1.03 \pm 0.09 \text{ mmol.l}^{-1}$, NS) whilst Controls displayed a slight decrease in fasting triglycerides (Pre: $0.82 \pm 0.08 \text{ mmol.l}^{-1}$; Post $0.73 \pm 0.06 \text{ mmol.l}^{-1}$, NS). Although the changes were not significant within the groups, these small changes resulted in a significant difference between

fasting triglycerides ($p < 0.05$) after the period of usual lifestyle. Offspring also displayed a significant increase in fasting GGT (Pre: $16.6 \pm 2.82 \text{ mmol.l}^{-1}$; Post $22.8 \pm 4.66 \text{ mmol.l}^{-1}$, $p < 0.05$) and although Controls did not display a significant change in fasting GGT (Pre: $12.28 \pm 1.90 \text{ mmol.l}^{-1}$; Post: $12.3 \pm 1.39 \text{ mmol.l}^{-1}$, NS) there was no significant difference between the groups after the period of usual lifestyle ($p = 0.06$). There were no significant changes in any indices of cardiorespiratory fitness over the course of the 7-week usual lifestyle period in either the Control or the Offspring volunteers (data not shown). However a small, but significant, increase in lean body mass in the Offspring (Pre: $44.7 \pm 1.7 \text{ kg}$; Post: $45.6 \pm 1.8 \text{ kg}$, $p < 0.05$) but not the Control (Pre: $42.8 \pm 1.7 \text{ kg}$; Post: $42.5 \pm 1.6 \text{ kg}$, NS) group was observed.

4.4.2 Compliance to exercise intervention

Six Offspring subjects and two Control subjects dropped out of the study before completion of the exercise intervention. Reasons for their withdrawal are shown in Figure 2.2. For subjects who completed the intervention and attended for post-intervention testing, Control subjects ($n = 34$) completed 28 ± 6 exercise sessions (out of 32 possible sessions), undertaking 1353 ± 58 minutes of exercise at an average heart rate of $141 \pm 2 \text{ beat.min}^{-1}$ ($73 \pm 1\%$ of maximum heart rate), over the 7-week intervention period. The Offspring subjects ($n = 28$) completed 29 ± 7 exercise sessions, undertaking 1451 ± 107 minutes of exercise at an average heart rate of $144 \pm 2 \text{ beat.min}^{-1}$ ($74 \pm 1\%$ of maximum heart rate). None of these values differed significantly between the Control and Offspring groups.

4.4.3 Post-intervention data

4.4.3.1 Physical characteristics and body composition

The Offspring group had small but statistically significant reductions in body mass (-1.6% , $p < 0.05$), total fat mass (-3.7% , $p < 0.01$), trunk fat mass (-3.0% , $p < 0.05$), leg fat mass (-2.8% , $p < 0.05$), arm fat mass (-6.9% , $p < 0.05$), waist circumference (-1.8% , $p < 0.01$), hip

circumference (-1.1%, $p < 0.05$), upper body (-4.5%, $p < 0.01$) and lower body fat (-2.8%, $p < 0.05$) following the exercise intervention, whereas in the Control group, the only statistically significant anthropometric changes were a small reduction in hip circumference (-0.9%, $p < 0.05$), upper body fat (3.1%, $p < 0.05$) and triceps skinfold (6.6%, $p < 0.01$). There was also a significant interaction for the change in body mass with the intervention between the Control and Offspring groups ($p < 0.05$), indicating a significantly greater reduction in body mass in response to the intervention the latter group (Table 4.1).

Table 4.1 Absolute changes in physical characteristics and body composition of Control and Offspring. Values are Mean \pm SEM.

	Controls	Offspring	P - value
	(n = 34)	(n = 28)	
Body mass (kg)	-0.15 \pm 0.28	-1.20 \pm 0.44*	< 0.05
Total fat mass (kg)	-0.58 \pm 0.23	-1.16 \pm 0.33**	0.14
Trunk fat mass (kg) ^a	-0.26 \pm 0.17	-0.63 \pm 0.25*	0.79
Leg fat mass (kg) ^a	-0.22 \pm 0.08	-0.28 \pm 0.13*	0.59
Arm fat mass (kg) ^a	-0.10 \pm 0.06	-0.33 \pm 0.13*	0.23
Android fat mass (kg)	-0.02 \pm 0.04	-0.04 \pm 0.05	0.74
Gynoid fat mass (kg)	-0.15 \pm 0.05	-0.04 \pm 0.10	0.27
Total lean mass (kg)	0.15 \pm 0.43	-0.04 \pm 0.28	0.73
Trunk lean mass (kg)	0.19 \pm 0.20	0.12 \pm 0.20	0.79
Leg lean mass (kg)	0.19 \pm 0.07	0.05 \pm 0.11	0.28
Arm lean mass (kg) ^a	0.04 \pm 0.05	-0.05 \pm 0.06	0.44
Upper Body Fat (kg) ^a	-0.37 \pm 0.21*	-0.96 \pm 0.25**	0.46
Lower Body Fat (kg) ^a	-0.22 \pm 0.08	-0.28 \pm 0.13*	0.59
Waist Circ (cm) ^a	-0.84 \pm 0.37	-1.61 \pm 0.45**	0.28
Hip Circ (cm) ^a	-0.94 \pm 0.32*	-1.15 \pm 0.41*	0.83
Biceps Skinfold (mm) ^a	0.74 \pm 0.65	-1.69 \pm 0.68	< 0.05
Triceps Skinfold (mm) ^a	-1.69 \pm 0.46**	-1.00 \pm 0.50	0.31
Subscapular Skinfold (mm)	-0.73 \pm 0.85	-0.79 \pm 0.60	0.96
Suprailiac Skinfold (mm) ^a	-1.26 \pm 0.64	1.68 \pm 1.62	0.24

Column p-values are for the interaction term in a 2-way ANOVA (group x trial) with repeated measures on the 'trial' factor. ^astatistical analysis performed on logarithmically transformed data. * p < 0.05, ** p < 0.01 for difference from baseline within group.

4.4.3.2 Concentrations of glucose, insulin, non-esterified fatty acid and 3-hydroxybutyrate

During the oral glucose tolerance test (OGTT) the Offspring group had significant reductions in fasting insulin (-17%, $p < 0.05$), mean insulin concentration during OGTT (-14%, $p < 0.01$), and change in concentration from fasting during the OGTT (-12%, $p < 0.01$). There was also a significant interaction for the change in insulin concentration during the OGTT ($p < 0.05$), and change from fasting ($p < 0.05$) with the intervention between the Control and Offspring groups, indicating a significantly greater reduction in circulating insulin in response to the intervention in the latter group (Table 4.2). The Offspring group also showed significant reductions in 3-OHB concentrations during the OGTT as measured by the mean concentration during OGTT (-49%, $p < 0.001$) and change in concentration from fasting ($p < 0.001$), whilst Controls showed a significant change in mean 3-OHB concentration during the OGTT (-34%, $p < 0.01$). There was also a significant interaction for the change in both mean concentration during OGTT ($p < 0.05$), and change from fasting during OGTT ($p < 0.01$) with the intervention between the Control and Offspring groups, indicating a significantly greater reduction in 3-OHB in response to the intervention in the Offspring group (Table 4.2).

Table 4.2 Absolute changes in glucose, insulin, NEFA and 3-OHB in the fasted state and during OGTT. Values are Mean \pm SEM.

	Controls (n = 34)	Offspring (n=28)	P - value
Glucose			
Fasting (mmol.l ⁻¹)	-0.08 \pm 0.08	-0.11 \pm 0.08	0.82
Mean during OGTT (mmol.l ⁻¹)	-0.08 \pm 0.18	-0.30 \pm 0.19	0.39
Change from fasting during OGTT (mmol.l ⁻¹)	-0.003 \pm 0.18	-0.20 \pm 0.17	0.46
Insulin			
Fasting ^a (mU.l ⁻¹)	-0.27 \pm 0.44	-1.81 \pm 0.62*	0.11
Mean during OGTT ^a (mU.l ⁻¹)	-3.78 \pm 2.86	-12.91 \pm 5.62**	< 0.05
Change from fasting during OGTT ^a (mU.l ⁻¹)	-3.51 \pm 2.79	-11.11 \pm 5.14**	< 0.05
NEFA			
Fasting (mmol.l ⁻¹)	-0.01 \pm 0.03	-0.01 \pm 0.03	0.93
Mean during OGTT ^a (mmol.l ⁻¹)	0.002 \pm 0.018	-0.003 \pm 0.012	0.52
Change from fasting during OGTT (mmol.l ⁻¹)	0.009 \pm 0.023	0.006 \pm 0.027	0.92
3-OHB			
Fasting ^a (mmol.l ⁻¹)	0.01 \pm 0.01	0.04 \pm 0.02	0.62
Mean during OGTT (mmol.l ⁻¹)	-0.02 \pm 0.01**	-0.05 \pm 0.01***	< 0.05
Change from fasting during OGTT (mmol.l ⁻¹)	-0.03 \pm 0.01	-0.09 \pm 0.02***	< 0.01

P-values are for the interaction term in a 2-way ANOVA (group x trial) with repeated measures on the ‘trial’ factor. ^astatistical analysis performed on logarithmically transformed data. * p < 0.05, ** p < 0.01, *** p < 0.001 for difference from baseline within group.

4.4.3.3 Insulin Sensitivity Index (ISI)

Offspring showed an increase of 23% (p < 0.01) in insulin sensitivity, whilst Controls increased ISI by 7% (NS). The interaction for the change in ISI was significant (p < 0.05)

suggesting a greater increase in ISI in Offspring in response to the exercise intervention (Figures 4.1-4.2).

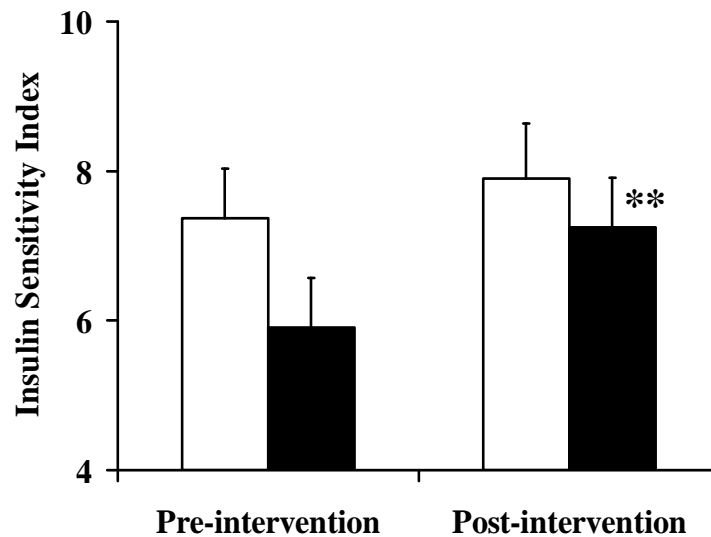


Figure 4.1 ISI in Controls (white bars) and Offspring (black bars) prior to, and after exercise intervention. ** $p < 0.01$ for within-group change from baseline.

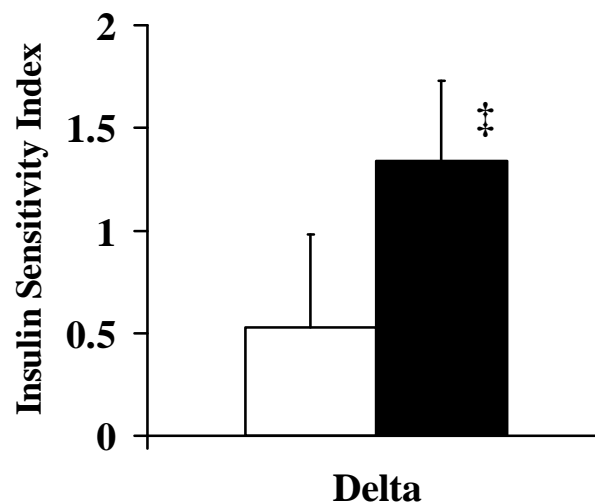


Figure 4.2 Magnitude of change in ISI in Controls (white bar) and Offspring (black bar) in response to exercise intervention. † $p < 0.05$ for between-group change.

4.4.3.4 Plasma lipids

No significant differences were observed in total cholesterol, LDL-cholesterol, HDL-cholesterol or triglycerides in either Controls or Offspring in response to the exercise intervention (data not shown).

4.4.3.5 Blood pressure and peripheral pulse wave velocity

Offspring displayed a trend towards reduction in diastolic blood pressure ($p = 0.07$) in response to the exercise intervention (Table 4.3). The interaction for the change in diastolic blood pressure was significant, suggesting that the exercise intervention produced different results in Controls compared to Offspring (Table 4.3). No significant changes were observed in fasting or post-glucose PWV in either group (Table 4.3).

Table 4.3 Absolute changes in fasting systolic and diastolic blood pressure and peripheral PWV in fasted, and 2-hour post-glucose states. Values are Mean \pm SEM.

	Controls (n=34)	Offspring (n=28)	P - value
Systolic blood pressure (mmHg)	-1.59 \pm 1.78	-3.36 \pm 2.27	0.54
Diastolic blood pressure (mmHg)	1.12 \pm 1.16	-3.43 \pm 1.50	< 0.05
Fasting PWV (m.s ⁻¹) ^a	-0.46 \pm 0.23	0.08 \pm 0.20	0.86
Post-glucose PWV (m.s ⁻¹) ^a	-0.21 \pm 0.20	-0.28 \pm 0.26	0.86

P-values are for the interaction term in a 2-way ANOVA (group x trial) with repeated measures on the 'trial' factor. ^astatistical analysis performed on logarithmically transformed data.

4.4.3.6 Liver enzymes

No significant differences in ALT or GGT were observed in either group after the intervention (data not shown).

4.4.3.7 Adipokines & C-reactive protein

Controls and Offspring had significant reductions in fasting adiponectin in response to exercise (Table 4.4). No significant difference was noted in the magnitude of the change in either group (Table 4.4). Controls displayed a significant reduction in IL-6 (-2.7%, $p < 0.05$). Offspring alone showed a significant reduction in fasting leptin in response to exercise, the interaction for the change between groups was also significant, suggesting a greater response due to exercise in Offspring (Table 4.4, Figures 4.3-4.4).

Table 4.4 Absolute change in fasting adipokines. Values are Mean \pm SEM.

	Controls (n=34)	Offspring (n=28)	P - value
Adiponectin ($\mu\text{g.ml}^{-1}$) ^a	-1.09 \pm 0.22^{***}	-0.94 \pm 0.34^{**}	0.48
Leptin (ng.ml^{-1}) ^a	-0.04 \pm 0.90	-5.3 \pm 1.4^{***}	< 0.01
TNF- α (pg.ml^{-1}) ^a	-0.02 \pm 0.06	0.01 \pm 0.04	0.92
IL-6 (pg.ml^{-1}) ^a	-0.43 \pm 0.16[*]	-0.08 \pm 0.08	0.20
Resistin (ng.ml^{-1})	0.33 \pm 0.34	-0.68 \pm 0.56	0.11
CRP (mmol.l^{-1}) ^a	-0.31 \pm 0.22	-0.01 \pm 0.33	0.10

P-values are for the interaction term in a 2-way ANOVA (group x trial) with repeated measures on the 'trial' factor. ^astatistical analysis performed on logarithmically transformed data. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for difference from baseline within group.

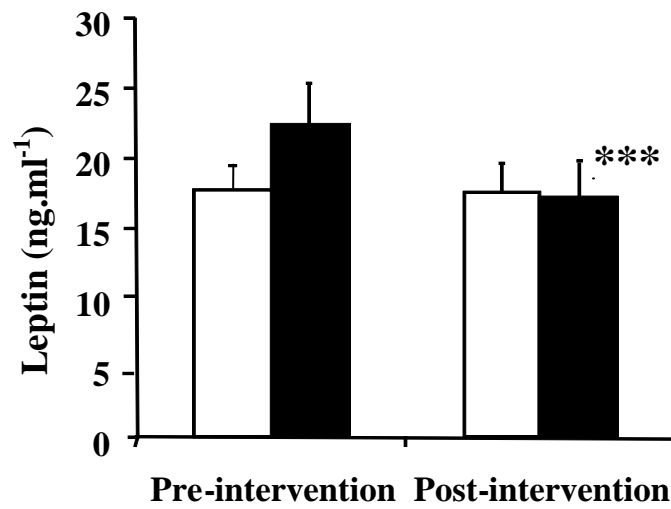


Figure 4.3 Leptin in Controls (white bars) and Offspring (black bars) prior to, and after exercise intervention. **p < 0.001 for within-group change from baseline.

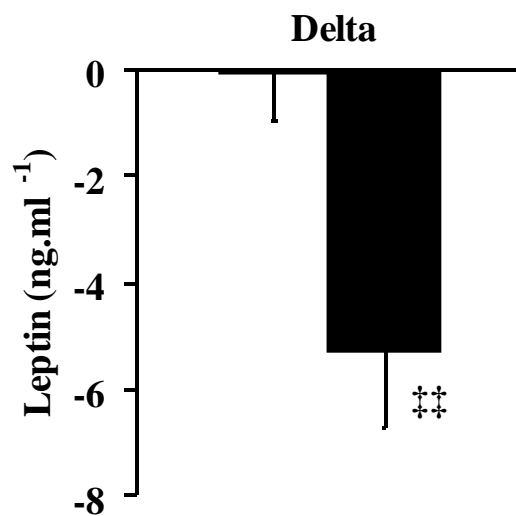


Figure 4.4 Magnitude of change in leptin in Controls (white bar) and Offspring (black bar) in response to exercise intervention. **p < 0.01 for between-group change.

4.4.3.8 Metabolic rate & substrate utilisation

Offspring displayed a significant reduction in respiratory exchange ratio (RER) in both fasted and post-glucose states, significant reductions in carbohydrate oxidation in both fasted and post-glucose conditions, and significantly increased fat oxidation in both states (Table 4.5). The interaction for the change between groups was also significant for these measurements, suggesting a greater response in the Offspring group. Overall metabolic rate did not change in either group, in either fasting or post-glucose conditions, after the exercise intervention.

Table 4.5 Absolute change in metabolic rate, respiratory exchange ratio (RER), rates of fat and carbohydrate (CHO) oxidation in Controls and Offspring in the fasted state and 2 hours after glucose load. Values are Mean \pm SEM.

	Controls (n=34)	Offspring (n=28)	P - value
Resting metabolic rate (kJ.kg ⁻¹ .min ⁻¹)	0.0002 \pm 0.0005	0.0006 \pm 0.001	0.93
Post-glucose metabolic rate (kJ.kg ⁻¹ .min ⁻¹)	0.001 \pm 0.0009	0.0006 \pm 0.0008	0.55
Fasting RER	-0.01 \pm 0.01	-0.05 \pm 0.01^{***}	< 0.01
Post-glucose RER	-0.02 \pm 0.01	-0.05 \pm 0.01^{***}	< 0.05
Fasting fat oxidation (mg.kg ⁻¹ .min ⁻¹)	0.09 \pm 0.07	0.41 \pm 0.08^{***}	< 0.01
Post-glucose fat oxidation (mg.kg ⁻¹ .min ⁻¹)	0.12 \pm 0.07	0.37 \pm 0.081^{***}	< 0.05
Fasting CHO oxidation (mg.kg ⁻¹ .min ⁻¹)	-0.17 \pm 0.16	-0.87 \pm 0.18^{***}	< 0.01
Post-glucose CHO oxidation (mg.kg ⁻¹ .min ⁻¹)	-0.14 \pm 0.19	-0.78 \pm 0.20^{***}	< 0.05

P-values are for the interaction term in a 2-way ANOVA (group x trial) with repeated measures on the 'trial' factor. ^astatistical analysis performed on logarithmically transformed data. * p < 0.05, ** p < 0.01, *** p < 0.001 for difference from baseline within group.

4.4.3.9 Cardiorespiratory fitness and habitual physical activity

Both Offspring and Controls significantly improved cardiorespiratory fitness as assessed by predicted $\dot{V}O_{2\max}$ (12% in the Controls and 15% in the Offspring, $p < 0.001$ for both) and by $\dot{V}O_2$ at lactate threshold (Table 4.6). The improvement in cardiorespiratory fitness in both groups with the intervention is also illustrated by the right-shift in the $\dot{V}O_2$ vs. heart rate relationship during sub-maximal exercise (Figure 4.5). The magnitude of improvement was similar in both groups, illustrated by a non-significant p-value for the interaction of group x trial.

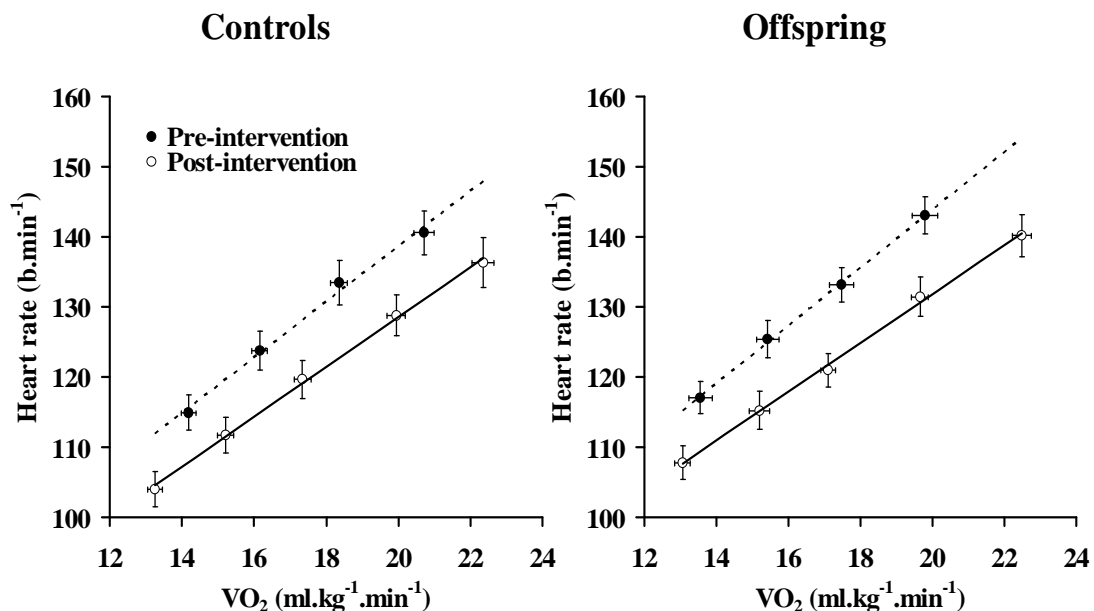


Figure 4.5. $\dot{V}O_2$ vs. Heart rate relationship pre- (black circles) and post-intervention (white circles) in Controls and Offspring.

The Control volunteers had a significant reduction in resting heart rate compared to their baseline values (6%, $p < 0.001$), whilst the Offspring group did not show a significant reduction (3%, NS) (Table 4.6). Neither Controls nor Offspring had a significant change in average daily heart rate compared to baseline however, although these changes were not

significant within the groups, the interaction for the change between groups was significant suggesting that the average daily heart rate had responded differently in Offspring compared to Controls (Table 4.6). Offspring alone showed a significant increase in habitual physical activity measured by the index of average heart rate – resting heart rate (Table 4.6). No significant differences were observed in time spent at heart rates indicative of light (1.5 times resting) and moderate (2 times resting) activity in either group, after adjustment for participation in the exercise intervention (Table 4.6).

Table 4.6 Absolute change in cardiorespiratory fitness and indices of habitual physical activity. Values are Mean \pm SEM.

	Controls (n=34)	Offspring (n=28)	P - value
$\dot{V}O_{2\max}^b$ (ml.kg ⁻¹ .min ⁻¹)	3.89 \pm 0.59^{***}	4.66 \pm 0.82^{***}	0.46
$\dot{V}O_2$ at lactate threshold (ml.kg ⁻¹ .min ⁻¹) ^{b,a}	2.84 \pm 0.49^{***}	2.37 \pm 0.71^{**}	0.59
Resting HR ^c (b.min ⁻¹)	-4.2 \pm 1.0^{***}	-2.5 \pm 1.1	0.27
Average daily HR (b.min ⁻¹)	-1.2 \pm 0.9	2.6 \pm 1.3	< 0.05
Average daily HR - resting HR	3.3 \pm 1.3	5.1 \pm 1.4^{**}	0.36
Time spent above 1.5 times resting HR (min.day ⁻¹) ^a	12.83 \pm 20.83	6.22 \pm 9.14	0.84
Time spent above 2 times resting HR (min.day ⁻¹)	2.08 \pm 4.10	4.86 \pm 2.06	0.55

P-values are for the interaction term in a 2-way ANOVA (group x trial) with repeated measures on the 'trial' factor. * p < 0.05, ** p < 0.01, *** p < 0.001 for difference from baseline within group. ^astatistical analysis performed on logarithmically transformed data. ^bEstimated from submaximal incremental treadmill test. ^cFrom pulse wave velocity determination. $\dot{V}O_2$: Oxygen uptake; $\dot{V}O_{2\max}$: Maximal oxygen uptake; HR: heart rate.

4.4.3.10 Energy intake and dietary composition

The only significant change in energy or macronutrient intake after the exercise intervention was a reduction (9%, $p < 0.05$) in protein intake in the Control group.

Table 4.7 Absolute change in energy and macronutrient intake. Values are Mean \pm SEM.

	Controls (n=34)	Offspring (n=28)	P - value
Energy (kJ.kg ⁻¹ .day ⁻¹)	-5.41 \pm 3.27	-5.70 \pm 4.60	0.96
Total fat (g. kg ⁻¹ .day ⁻¹)	-0.01 \pm 0.04	-0.05 \pm 0.05	0.56
SFA (g. kg ⁻¹ .day ⁻¹) ^a	-0.01 \pm 0.02	-0.01 \pm 0.02	0.94
MUFA (g. kg ⁻¹ .day ⁻¹)	-0.01 \pm 0.02	-0.02 \pm 0.02	0.53
PUFA (g. kg ⁻¹ .day ⁻¹) ^a	-0.01 \pm 0.01	-0.03 \pm 0.02	0.59
Total CHO (g. kg ⁻¹ .day ⁻¹) ^a	-0.18 \pm 0.15	-0.29 \pm 0.15	0.60
Sugar (g. kg ⁻¹ .day ⁻¹) ^a	-0.17 \pm 0.11	-0.21 \pm 0.10	0.74
Starch (g. kg ⁻¹ .day ⁻¹) ^a	-0.10 \pm 0.07	0.0001 \pm 0.10	0.23
Protein (g. kg ⁻¹ .day ⁻¹) ^a	-0.10 \pm 0.03 [*]	-0.07 \pm 0.05	0.58

P-values are for the interaction term in a 2-way ANOVA (group x trial) with repeated measures on the 'trial' factor. ^astatistical analysis performed on logarithmically transformed data. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for difference from baseline within group.

4.4.4 Chronic effects of exercise intervention

Volunteers randomised to the Exercise group had repeated metabolic assessment 3 days after the final exercise session (section 4.2.2). Volunteers had been asked to avoid any further planned exercise during this period to determine which metabolic variables might be acutely influenced by exercise, and which of the metabolic changes observed might persist in response to the cumulative effects of the intervention.

4.4.4.1 Three-day ‘detraining’ results

Insulin sensitivity did not change significantly in Offspring or Controls after 3 days without planned exercise (Table 4.8). Offspring did show a significant reduction in fasting 3-OHB concentration (-43%, $p < 0.001$) and the interaction for this change between the groups was also significant (Table 4.8). This would suggest a reduction in fasting hepatic fat oxidation, and this suggestion is supported by the observed significant reductions in fat oxidation in both fasting, and post-glucose conditions, with concurrent increases in respiratory exchange ratio (RER) in both states in Offspring alone (Table 4.8). The interaction for the change in fasting fat oxidation between the groups is also significant, suggesting that 3 days of detraining has a different effect on fasting fat oxidation in Offspring compared to Controls.

The only other significant metabolic variable to change with detraining was the fasting leptin concentration, which was significantly increased in Offspring (33%, $p < 0.001$). Fasting leptin concentration in the Control group did not alter significantly (7%, NS) (Table 4.8). The interaction for the change in leptin between groups was significant, suggesting a differing response to detraining in Offspring compared to Controls (Table 4.8).

Table 4.8 Absolute change in metabolic variables in Controls and Offspring after 3 days of ‘detraining’. Values are Mean \pm SEM.

	Controls (n=19)	Offspring (n=17)	P - value
ISI ^a	-0.16 \pm 0.67	0.14 \pm 0.94	0.64
Fasting 3-OHB (mmol.l ⁻¹) ^a	-0.021 \pm 0.017	-0.068 \pm 0.020^{***}	< 0.05
Fasting RER	0.02 \pm 0.01	0.05 \pm 0.01^{**}	0.08
Post-glucose RER	0.01 \pm 0.01	0.03 \pm 0.01[*]	0.08
Fasting fat oxidation (mg.kg ⁻¹ .min ⁻¹)	-0.12 \pm 0.10	-0.42 \pm 0.09^{***}	< 0.05
Post-glucose fat oxidation (mg.kg ⁻¹ .min ⁻¹)	-0.086 \pm 0.048	-0.245 \pm 0.092^{**}	0.10
Fasting CHO oxidation (mg.kg ⁻¹ .min ⁻¹)	0.29 \pm 0.22	0.87 \pm 0.25^{**}	0.11
Post-glucose CHO oxidation (mg.kg ⁻¹ .min ⁻¹)	0.086 \pm 0.13	0.514 \pm 0.23[*]	0.82
Leptin (ng.ml ⁻¹)	0.19 \pm 0.80	5.80 \pm 2.15^{***}	< 0.01

P-values are for the interaction term in a 2-way ANOVA (group x trial) with repeated measures on the ‘trial’ factor. ^astatistical analysis performed on logarithmically transformed data. * p < 0.05, ** p < 0.01, *** p < 0.001 for difference from immediate post-intervention testing within group.

4.5 Discussion

The main finding of this study was that women with a family history of type 2 diabetes significantly improved insulin sensitivity in response to an exercise training programme, whereas no significant change in insulin sensitivity was observed in women matched for age and BMI but with no diabetes family history. ISI was 22% lower ($p < 0.05$) in the Offspring than the Controls group at baseline, but only 8% (NS) lower following exercise training. This augmented response to exercise training in the Offspring supports the primary hypothesis and is consistent with the literature from cross-sectional studies (Ahn et al. 2004; Gill & Malkova 2006; Higgins et al. 2005). Changes in body composition over the intervention were small in both groups, although the Offspring lost slightly more body fat than the Controls (by ~0.6 kg). The larger increase in ISI in the Offspring in response to the exercise intervention could not be explained by differences in compliance to the exercise intervention, or differences in changes in energy intake, or fitness between the Control and Offspring groups.

Although compliance with the intervention was not significantly different between the groups there remains a possibility that volunteers could have altered their habitual activity levels and that this could have influenced insulin sensitivity. The improvement in cardiorespiratory fitness in both groups was similar. Average daily heart rate did not change significantly in either group, however because the Controls decreased their average heart rate (-1%, NS) and Offspring increased their average heart rate (3%, NS) these changes were found to be statistically significant between the groups. This might suggest an increase in habitual activity in the Offspring group, however other indices of physical activity, such as the difference between average heart rate and resting heart rate, and the time spent at 1.5 times and 2 times resting heart rates (crude markers of time spent at heart rates consistent with 'light' and 'moderate' activity) were not different between Controls and Offspring. Thus, no evidence exists for a significant change in habitual activity levels outwith participation in the intervention, although it is accepted that the indices of physical activity are relatively crude.

Despite having similar levels of adiposity and fitness, and similar energy and macronutrient intakes, the Offspring group were more insulin resistant than the Control group at baseline, consistent with other data in the literature (Kriketos et al. 2004;Nyholm et al. 2004;Perseghin et al. 1997). It is possible that this increased baseline insulin resistance contributed to the Offspring group's greater change in insulin sensitivity in response to the intervention, since other studies have reported greater exercise-induced changes in insulin sensitivity in those who are more insulin resistant at baseline (An et al. 2005;Gill & Malkova 2006;Magkos et al. 2007) . As Offspring have a genetic predisposition to increased insulin resistance and thus increased baseline insulin resistance is a well-established component of the Offspring 'phenotype' (Kriketos et al. 2004;Nyholm et al. 2004;Perseghin et al. 1997), it is difficult to determine the extent to which diabetes family history versus increased baseline insulin resistance itself contributed to the increased change in ISI with exercise training in the present study. However, irrespective of whether this is due to increased insulin resistance at baseline or other underlying factors, the present data indicate that Offspring form an easily identifiable group who will particularly benefit from increasing their level of physical activity.

Circulating leptin concentrations decreased by 24% in response to the intervention in the Offspring but were unchanged in the Control group. Leptin concentrations correlate with fat mass (Correia & Rahmouni 2006), and a change in the Offspring group might have been expected since fat mass was reduced. However, a 'rebound' effect on fasting leptin in Offspring alone was observed after 3 days of 'detraining' and therefore, the metabolic effect of exercise on leptin cannot solely be attributed to a change in fat mass, since body mass did not change over these 3 days. The reduction in leptin concentrations in the Offspring group may reflect an increase in 'leptin sensitivity'. Normally, leptin promotes lipid oxidation by increasing PPAR α expression (Lee et al. 2002). Thus, it has been proposed that leptin

resistance leads to insulin resistance by causing ectopic accumulation of lipids in non-adipose tissues, such as skeletal muscle, the liver and the pancreas (Correia & Rahmouni 2006). Indeed, a recent study reported a significant correlation between changes in circulating leptin concentrations and changes in intramuscular lipid content following an exercise intervention in older obese adults with impaired glucose tolerance (Solomon et al. 2008) and significant correlations between changes in insulin concentrations and changes in leptin concentrations following weight loss interventions have been reported (Carantoni et al. 1999; Doucet et al. 2000). In this study, Offspring alone displayed significant exercise mediated increases in rates of fat oxidation, and after 'detraining' significant reduction in fasting fat oxidation. Therefore, in the Offspring group alone, reduced leptin concentration, increased fat oxidation and increased insulin sensitivity were found after the exercise intervention. Also, consistent with a possible causal relationship between leptin, fat oxidation and insulin sensitivity, in this group alone the improvement in both fasting leptin concentration and fat oxidation were rapidly reduced after a period without exercise, and these changes were not accompanied by a significant change in insulin sensitivity. Thus, improved leptin sensitivity in the Offspring could have contributed to their increased insulin sensitivity following the intervention, and may signify, or even mediate, a subsequent reduction in insulin sensitivity which has been previously observed with cessation of exercise (Liu et al. 2008). It is however unclear from the present data why the exercise intervention only influenced leptin concentrations in the Offspring. Differential effects of exercise-training on adipose tissue expression of leptin and its receptor between the Offspring and Controls may have contributed, and this warrants further investigation.

These results differ slightly from the findings of earlier smaller scale studies which found similar improvements in insulin sensitivity in response to an exercise training intervention in adults with and without a family history of type 2 diabetes (Ostergard et al. 2006; Perseghin et al. 1996). One possible explanation for this discrepancy relates to the volume of exercise undertaken. In the progressive exercise intervention in the present study, subjects performed

300 minutes of exercise per week during the final two weeks of the intervention, compared to 135-180 minutes of exercise per week in the previously published reports (Ostergard et al. 2006;Perseghin et al. 1996). In an evolutionary context, even three hours of exercise per week represents a relatively low volume (Eaton & Eaton 2003), and it is possible that improvements in insulin sensitivity with exercise would plateau at higher exercise volumes in those with a family history of diabetes compared with those with no diabetes family history (Gill & Malkova 2006). Thus, in the earlier reports (Ostergard et al. 2006;Perseghin et al. 1996), the training stimulus, while sufficient to induce improvements in insulin sensitivity, may not have been great enough to induce optimal benefits in the group with a diabetes family history. Further study is needed to establish the dose-response relationship between physical activity and insulin sensitivity in those with a diabetes family history, which has potential implications for physical activity guidelines.

An additional explanation for the influence of exercise in this study is the sex of the participants. The earlier reports (Ostergard et al. 2006;Perseghin et al. 1996) studied mixed groups of men and women, whereas the current study involved only women. In the HERITAGE family study, the improvement in insulin sensitivity in response to exercise training was three times as great in men as in women (16% vs. 5%) (Boule et al. 2005), suggesting that changes in insulin sensitivity differ between the sexes. Thus, it is possible that the augmented response to exercise training in the Offspring group in the present study is sex-specific and further investigation is required to determine whether these findings extend to men.

The present investigation has a number of strengths. It is the largest study to date investigating the effects of exercise training on insulin sensitivity in Offspring of patients with type 2 diabetes, and thus well-powered to detect changes in the main outcome measures, and the exercise intervention was tightly controlled and quantified. The Control and

Offspring groups were well-matched for age and adiposity. Diet was not strictly controlled during the intervention, however subjects were asked not to change their dietary habits during the study and subjects undertook 'gold-standard' 7-day weighed food records during the weeks preceding each OGTT: these data indicate that dietary intakes did not differ between the groups or change over the course of the intervention. It is always difficult to undertake intervention studies in pre-menopausal women because of the potentially confounding effects of menstrual cycle. However, it is important to examine this group because they are relatively understudied in the field of cardiovascular and diabetes risk and because the development of diabetes in women has a more profound effect on cardiovascular risk than in men (Mak & Haffner 2003). Attempts to control for the effects of menstrual cycle were made by performing pre- and post-intervention assessments at an interval of 8 weeks, so that, as far as possible, all measurements for each individual would be made in the same menstrual cycle phase (Creinin, Keverline, & Meyn 2004). Adopting this approach does mean that women with very long or short menstrual cycles may not have been studied in the same phase of menstrual cycle in their pre- and post-intervention tests, but the number of women to which this would apply would have been small (Creinin, Keverline, & Meyn 2004) and this is unlikely to have had a major influence on the study findings.

This study is not without limitations. Insulin sensitivity was assessed using OGTTs, rather than 'gold standard' euglycaemic hyperinsulinaemic clamps. The reason for this was pragmatic; over 160 OGTTs were performed over the course of this study and it would not have been feasible to undertake a study of this size using euglycaemic clamps. However, the ISI index used in this study correlates well with clamp-derived measures of insulin sensitivity (20) and has been widely used in the literature (915 citations from April 2000 to November 2009, ISI Web of Knowledge, <http://apps.isiknowledge.com>, accessed 11 November 2009).

In conclusion, the data from the present study indicate that women with a family history of type 2 diabetes experience greater improvements in insulin sensitivity following an exercise intervention than women with no diabetes family history undertaking the same programme. Further study is needed to elucidate the mechanisms underpinning why Offspring exhibited this augmented response to exercise training and to determine the dose-response relationship for exercise-induced changes to insulin sensitivity in those with and without a family history of type 2 diabetes, which could potentially lead to refinement of physical activity guidelines for the improvement of insulin sensitivity and prevention of diabetes.

CHAPTER 5

DETERMINANTS OF THE EXERCISE-MEDIATED CHANGE IN INSULIN SENSITIVITY

5.1 Introduction

Exercise has been shown to improve insulin sensitivity in the obese (DeFronzo, Sherwin, & Kraemer 1987), the overweight (Nassis et al. 2005), normal weight subjects (Schafer et al. 2007) and in patients with type 2 diabetes (Christ-Roberts et al. 2004; Winnick et al. 2008). Additionally, exercise has been of benefit in preventing diabetes in patients with impaired glucose tolerance (Knowler et al. 2002; Pan et al. 1997; Tuomilehto et al. 2001; Eriksson et al. 1991; Ramachandran et al. 2006) and normal glucose tolerance (Davey et al. 2005) and has been shown to be of greater benefit to insulin sensitivity in pre-menopausal women with a diabetes family history (Offspring) than age and BMI matched controls (Controls) (see Chapter 4). The latter improvement was seen despite a similar exercise ‘dose’ and similar improvement in cardiorespiratory fitness. However, changes in adiposity and adipose tissue hormone concentrations, particularly leptin, were observed in the Offspring group. Additionally, exercise appeared to influence fat oxidation to a greater extent in Offspring compared to Controls, and the Offspring alone displayed increased circulating leptin, and decreased fat oxidation (measured by both fasting 3-OHB concentration and by measurements of indirect calorimetry) after three days of exercise avoidance. Since leptin is strongly correlated with fat mass (Considine et al. 1996), and decreased fat oxidation is also associated with overweight and obesity (Lillioja et al. 1986), it could be argued that the acute changes in these factors occurred due to the change in body fat with exercise and that this simply accompanies, rather than influences the change in insulin sensitivity. However, this does not explain the changes in fat oxidation, 3-OHB and leptin observed after 3 days of detraining, which occurred in the absence of changes in body mass.

Reduction in fat mass is recognised to correlate with improved insulin sensitivity (Ross et al. 2000). The mechanisms underpinning this improvement are multifactorial and the mechanism by which fat loss is achieved is also likely to be important. To illustrate this point, Giugliano and colleagues (2004) found improved insulin sensitivity and reduced inflammatory markers in obese women who had lost an average of 2.7 kg of fat after liposuction (Giugliano et al. 2004). Klein and co-workers (2004) did not find improvements in metabolic health represented by increased insulin sensitivity or reduced inflammation after liposuction fat loss of around 10.5 kg (Klein et al. 2004).

However, reduction in fat mass by dietary restriction, exercise or bariatric surgery is consistently associated with reduced markers of inflammation (Zahorska-Markiewicz et al. 2008; Ryan & Nicklas. 2004; Murri et al. 2010). Reduced fat mass is also associated with increased circulating adiponectin (Polak et al. 2006) which is considered to have systemic anti-inflammatory effects, promoting insulin signalling and lipid oxidation, reduction in ectopic fat deposition and increasing NO bioavailability (Sowers 2008). Therefore through a reduction in inflammatory proteins such as TNF α and increase in adiponectin, endothelial dysfunction in skeletal muscle capillary beds and impaired insulin signalling might be ameliorated (Ziccardi et al. 2002). Reductions in circulating leptin are observed with reduced fat mass (Considine et al. 1996) and some groups have suggested that after weight loss, the reduction in leptinaemia and the associated increase in fat oxidation is evidence of improved 'leptin sensitivity' (Solomon et al. 2008). However, although mechanisms have been described which link the action of leptin to skeletal muscle fat oxidation in health and the development of apparent 'leptin resistance' in states of obesity and insulin resistance (Dyck 2009), none have yet been described to delineate cause and effect in the circumstance of improved metabolic health after weight loss. Reduced fat mass is also associated with alterations in lipid metabolism which would be expected to improve insulin sensitivity. Reduced lipolysis and increased skeletal muscle fat oxidation are reported after weight loss due to caloric restriction alone and caloric restriction plus exercise (Savage, Petersen, &

Shulman 2007;Schenk et al. 2009). Thus, reduced fat mass is also likely to improve insulin sensitivity by reducing exposure of skeletal muscle to lipid intermediates as a result of decreased fatty acid uptake and enhanced oxidation of intramyocellular lipid.

Aerobic exercise interventions are frequently associated with both improvements in insulin sensitivity, and reductions in adiposity (Boudou et al. 2003;Kim et al. 2007;Polak et al.2006;Solomon et al. 2008). However, not all exercise interventions which induce improved insulin sensitivity are associated with decreased adiposity (Nassis et al. 2005;Ostergard 2006). Therefore fat loss *per se* may not be the key determinant of exercise mediated increases in insulin sensitivity. One aim of this study was therefore to determine the role of body composition changes in mediating exercise-associated changes in insulin sensitivity.

Insulin sensitivity may be altered in adipose tissue, liver and/or skeletal muscle. However in the context of an exercise mediated increase in insulin sensitivity, the predominant target tissue is likely to be skeletal muscle (Corcoran et al. 2007;DeFronzo et al. 1981;Shulman et al. 1990), resulting in both increased insulin-dependent, and independent glucose uptake (Hawley & Lessard 2008). The final common pathway in this process appears to be increased translocation of the GLUT4 transporter (although it is unlikely that exercise mediates improved insulin sensitivity entirely via this pathway) (O'Gorman et al. 2006). As described in Section 1.16.1, a single exercise session produces acute effects on skeletal muscle which persist for up to 4 hours. These include contraction-induced GLUT4 translocation, mediated by activation of CaMK, increased capillary recruitment producing enhanced glucose delivery to muscle and may activate AMPK to promote GLUT4 cycling (Wojtaszewski & Richter 2006). Beyond 4 hours IRS-2 activity is increased and downstream atypical PKC activity mediates further GLUT4 translocation (Sakamoto & Goodyear 2002;Zierath et al. 2000;Howlett et al. 2006) and glycogen depletion is likely to stimulate AMPK further (Jensen, Wojtaszewski, & Richter 2009; McBride & Hardie. 2009). Although the proximal

parts of the insulin signalling cascade do not appear to be as acutely sensitive to exercise as the downstream components, there is some evidence from *in vitro* studies that after exercise, GLUT4 cycles to areas of the myocyte which are more insulin sensitive, leading to more GLUT4 translocation in response to insulin (Geiger et al. 2006).

With respect to aerobic training, both seven day, short duration intervention (O’Gorman et al. 2006) and chronic endurance regimes (Yu et al. 2001) have shown alteration in the expression of components of the insulin signalling cascade which are not thought to be directly responsible for improved insulin sensitivity (Yu et al. 2001; O’Gorman et al. 2006). Short term training has not shown consistent increases in expression of key components of the insulin signalling cascade although insulin receptor activity may be increased (Wojtaszewski & Richter 2006; Youngren et al. 2001) however, chronic training does increase expression of the insulin receptor, Akt and GLUT4 and glycogen synthase (Wojtaszewski & Richter 2006). Chronic exercise training may induce increased insulin sensitivity indirectly, by ameliorating pathological processes such as intramyocellular lipid accumulation and chronic inflammation. Increased lipid oxidation in exercised myocytes leads to reduced IMCL and intermediates (Goodpaster et al. 2003) (ceramides, diacylglycerol (DAG) and long-chain fatty acids) leading to improved insulin signalling by increased Akt and reduced PKC activity (Bonen, Dohm, & van Loon 2006)(Delarue & Magnan 2007; Turcotte & Fisher 2008; Wolf 2008). Alterations in muscle structure and function are likely to improve insulin sensitivity as fibres ‘switch’ to more oxidative type and increase capillary density (Daugaard & Richter 2001). Exercise-mediated increases in circulating adiponectin and local expression of adiponectin receptors may have direct effects on muscle lipid oxidation and indirect effects on inflammation and endothelial function (Kim et al 2007; Vu et al 2007; Sowers 2008). Therefore, whilst a sedentary lifestyle may predispose to insulin resistance partly through disrupted insulin signalling, exercise may induce improved insulin sensitivity by methods which circumvent, rather than reverse, these changes (Turcotte & Fisher 2008; O’Gorman et al. 2006).

It is difficult to generalise the role of adipose tissue associated factors (such as circulating fatty acid concentrations, rates of substrate utilisation, and adipokine concentrations) in the exercise-mediated change in insulin sensitivity, given the heterogeneity of the research methods used thus far. Variations in study subjects and training regimes in particular often provide apparently conflicting information. Polak and colleagues (2006) described an aerobic training related improvement in insulin sensitivity associated with reduced serum leptin in obese females (Polak et al 2006). However the same group reported improved insulin sensitivity without any change in adipokines in obese men participating in a resistance-type training programme (Klimcakova et al. 2006). Similarly, adiponectin has not been consistently associated with exercise mediated improvements in insulin sensitivity (Boudou et al. 2003; Nassis et al. 2005; Hulver et al. 2002; Yatagai et al. 2003) in groups ranging from lean subjects to middle aged men with type 2 diabetes. A further aim of the current study was therefore to examine the contribution of adipose tissue derived hormones to an exercise-mediated change in insulin sensitivity. Additionally, since insulin resistance is characterised by reduced skeletal muscle fatty acid oxidation this study also aimed to assess the associations between whole body fat oxidation and the change in insulin sensitivity after the exercise intervention.

The aim of this study was therefore to examine the interactions between the changes in metabolic, cardiorespiratory, lifestyle and anthropometric factors and the change in insulin sensitivity, in an attempt to determine which processes contribute to the change in insulin sensitivity seen following a seven-week aerobic training programme in sedentary pre-menopausal women. Additionally, by identification of the factors which contribute to an exercise-mediated change in insulin sensitivity, this study aimed to describe possible metabolic pathways which might explain these changes. In view of the alterations in adiposity and circulating adipose tissue hormone concentrations in response to exercise, the

interaction between the change in these variables and the change in insulin sensitivity was of particular interest. Finally, this study aimed to determine whether exercise improves insulin sensitivity by different mechanisms in Offspring compared to Controls, or whether the fundamental difference in exercise response is mediated by the same metabolic pathway which is more responsive to exercise in the Offspring group.

5.2 Methods

5.2.1 Volunteers

Volunteers were recruited and screened as detailed in section 2.1. Specific inclusion criteria are defined in section 2.1.3. Further information detailing recruitment response and excluded volunteers can be found in Figure 2.2. The Offspring group was composed of women with a first-degree relative with T2D. The Control group was composed of women with no first or second degree relative with T2D. This study was conducted on twenty eight Offspring and thirty four Control volunteers. Baseline volunteer characteristics are described in Section 4.2.1.

5.2.2 Study Design

Volunteers were randomised into ‘Exercise’ or ‘Delayed-Exercise’ intervention arms using a computer programme. The study design is shown in Figures 2.3-2.4 (Section 2.1.3). Metabolic, anthropometric and cardiorespiratory testing (Section 2.2-2.3, 2.6) was performed at baseline and after a progressive 7-week exercise intervention (Section 2.7). Volunteers were studied at eight-week intervals to allow, as far as possible, for all metabolic, fitness and body composition assessments for each individual to be made in the same menstrual cycle phase (Creinin, Keverline, & Meyn 2004). Volunteers were asked not to alter their dietary habits during their participation in the study. Evaluation of both diet and habitual physical activity was performed at baseline and during the last week of the intervention, as described in sections 2.8 - 2.9.

5.2.3 Fitness testing

Cardiorespiratory fitness was determined by calculation of predicted $\dot{V}O_{2\max}$ and determination of the lactate threshold, as described in section 2.6

5.2.4 Metabolic testing

Volunteers attended for metabolic testing as described in Section 2.3. Assessment of metabolic rate, substrate utilisation, blood pressure and PWV (Section 2.3.2 – 2.3.5) was made in the fasted state and 2 hours after a 75 g oral glucose tolerance test (Section 2.3.7). Fasting plasma samples were analysed for insulin, glucose, NEFA, 3-OHB, leptin, adiponectin, resistin, CRP, IL-6, $TNF\alpha$, ALT, GGT, total cholesterol, HDL-cholesterol, and triglycerides as described in Section 2.4. During the OGTT plasma samples were obtained at 30, 60, 90 and 120 minutes and analysed for insulin, glucose, NEFA and 3-OHB (Section 2.4). Mean concentrations of glucose, insulin, NEFA and 3-OHB during the OGTT were calculated as described in section 3.2.9.1. Insulin sensitivity was expressed as the Insulin Sensitivity Index (Matsuda & DeFronzo 1999) and calculated as described in section 2.11.3.

5.2.5 Body composition assessment

DEXA scans were used to determine body composition and fat distribution (section 2.2.5). Height, body mass, waist and hip circumferences, and biceps, triceps, suprailiac and subscapular skinfolds were also determined using standard protocols (section 2.2) (Marfell-Jones et al. 2006).

5.2.6 Exercise intervention

Volunteers underwent a 7-week endurance-type exercise training programme as described in Section 2.7.

5.3 Statistical analysis

Data were analysed using Statistica (version 6.0, StatSoft Inc., Tulsa, Oklahoma) and Minitab (version 13.1, Minitab Inc., State College, Pennsylvania). Prior to analysis, all data were tested for normality using the Anderson-Darling normality test and if necessary, logarithmically transformed. Associations between variables were assessed using univariate linear correlations. To determine the extent to which variables were related to change in insulin sensitivity index independently of other variables, univariate linear regressions were then performed between the residuals for change in insulin sensitivity index of the regression between change in insulin sensitivity index (ISI) and the other variables as previously described in Section 3.2.9.2. Finally, to determine which variables were independent predictors of the change in ISI, multiple-regression analysis was performed. Statistical significance was accepted at the $p \leq 0.05$ level.

5.4 Results

5.4.1 Major influences on exercise mediated change in insulin sensitivity

Exercise-mediated insulin sensitivity improved to a greater extent in the Offspring group compared to Controls, although there was inter-individual variability within each group (Figure 5.1). This improvement was accompanied by significant increases in fat oxidation (as measured by 3-hydroxybutyrate concentrations (Williamson & Whitelaw 1978) in response to oral glucose, and by evaluation of the rate of fat oxidation by indirect calorimetry), and significant reductions in body mass, fat mass and circulating leptin (Section 4). These changes were observed in Offspring alone, despite similar adherence to the exercise regime, energy expended during exercise and similar improvements in cardiorespiratory fitness.

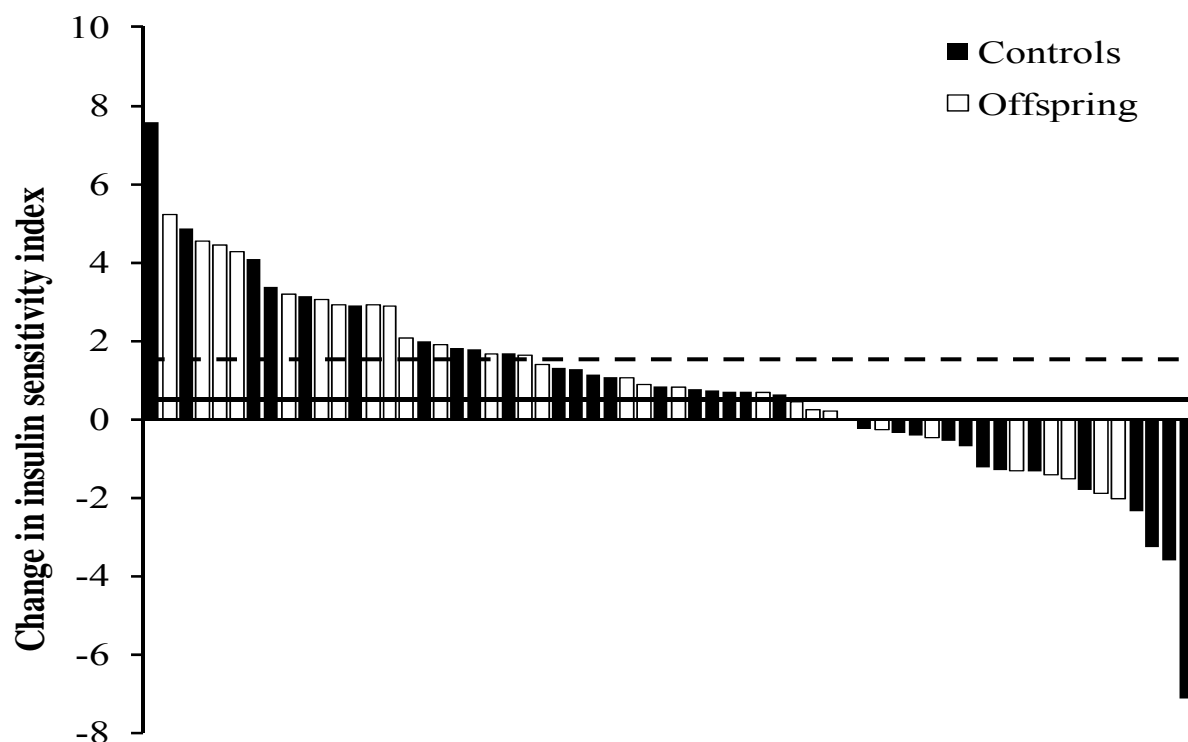


Figure 5.1 Individual changes in insulin sensitivity index (ISI) in whole group (n = 62) after exercise intervention. Volunteers ranked in order of change in unadjusted ISI. Horizontal lines indicate mean change in ISI in Controls (solid line) and Offspring (dotted line).

5.4.2 Baseline influences on exercise-mediated change in insulin sensitivity

The change in insulin sensitivity in response to the exercise intervention did not correlate with baseline anthropometric or behavioural variables, but was significantly negatively associated with baseline insulin sensitivity (Figure 5.2) and with diabetes family history (with 0 and 1 included as dummy variables for negative and positive diabetes family history, respectively) in the combined group analysis. In addition, resting and post-glucose metabolic rate were positively correlated with the exercise-mediated increase in insulin sensitivity (Table 5.1).

When analysed separately, Controls displayed a positive correlation between baseline post-glucose metabolic rate and change in insulin sensitivity and a negative correlation between baseline CRP and change in insulin sensitivity (Table 5.1). In Offspring, baseline insulin sensitivity alone was negatively associated with the change in insulin sensitivity after exercise (Figure 5.2).

Figure 5.2 Scattergram showing relationship between baseline ln ISI and change in ln ISI.

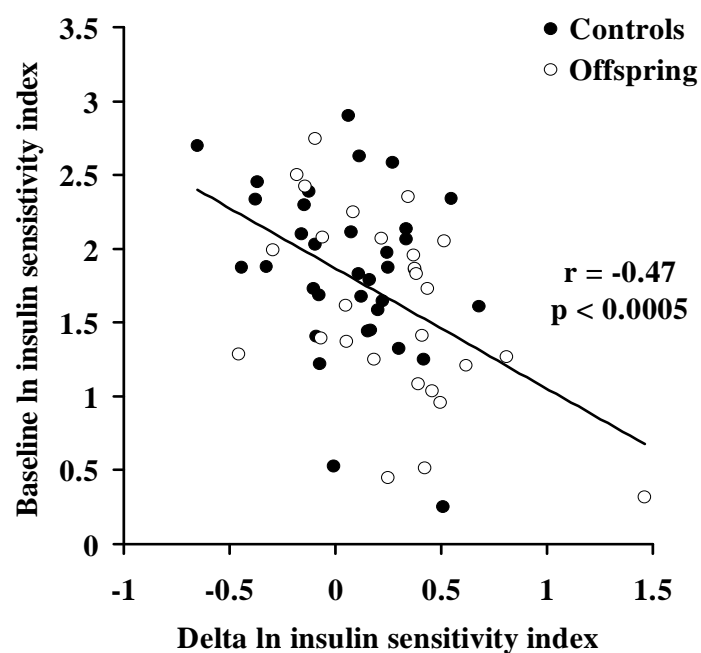


Table 5.1 Correlations between baseline variables and change in ISI in response to exercise training in Combined, Control and Offspring groups.

	Variable	Correlations with change in insulin sensitivity		
		Combined	Controls	Offspring
Family history variables	Diabetes family history	0.27 (< 0.05)	-	-
Body composition variables	Body Mass	0.10 (0.45)	0.17 (0.36)	-0.03 (0.87)
	Total fat mass	0.13 (0.36)	0.25 (0.19)	-0.07 (0.75)
Physiological/metabolic variables	$\dot{V}O_{2\max}$	-0.01 (0.94)	0.01 (0.94)	0.12 (0.55)
	RMR	0.29 (< 0.05)	0.17 (0.34)	-0.02 (0.91)
	Post-glucose MR	0.27 (< 0.05)	0.37 (< 0.05)	-0.16 (0.42)
Behavioural variables	Energy intake	0.01 (0.96)	0.23 (0.22)	-0.23 (0.25)
	Average HR – Resting HR	-0.25 (0.06)	-0.17 (0.40)	-0.20 (0.32)
	Resting HR	0.13 (0.33)	-0.02 (0.94)	0.20 (0.31)
Biochemical variables	ISI ^a	-0.47 (< 0.0005)	-0.33 (0.06)	-0.53 (< 0.005)
	Leptin ^a	0.07 (0.61)	-0.16 (0.37)	0.18 (0.35)
	CRP ^a	-0.01 (0.95)	-0.35 (< 0.05)	0.35 (0.07)
	TNF α ^a	0.01 (0.96)	-0.09 (0.63)	0.18 (0.36)
	adiponectin ^a	-0.15 (0.26)	0.02 (0.92)	-0.33 (0.09)

n = 62, values are correlation coefficients with p-values in brackets. ^aStatistical analysis performed on logarithmically transformed data. Significant correlations in bold.

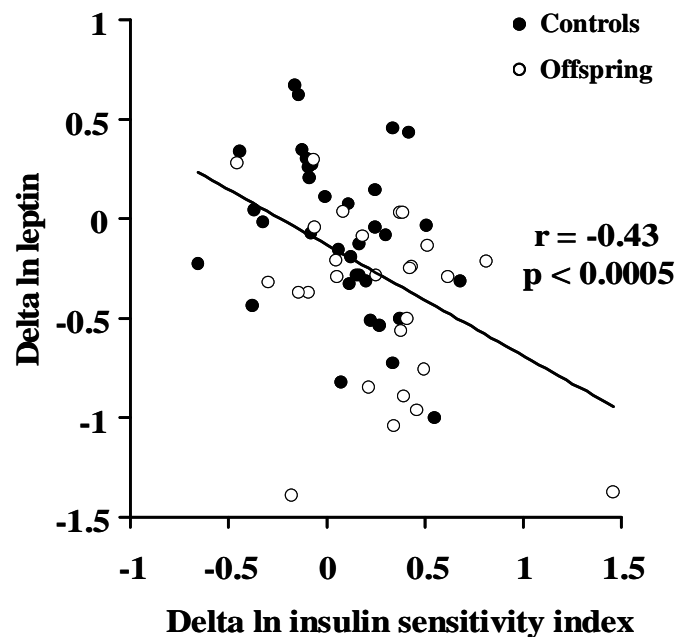
5.4.3 Exercise mediated changes influencing insulin sensitivity

When examined as a combined group, the exercise-mediated improvement in insulin sensitivity was associated with a reduction in circulating leptin (Figure 5.3). The change in insulin sensitivity in response to exercise also correlated significantly with a reduction in resting heart rate and an increase in the surrogate of habitual physical activity, average daily heart rate minus resting heart rate (Table 5.2). Additionally, improved exercise-mediated insulin sensitivity was associated with reduced lower body fat mass and hip circumference and was positively correlated with the net energy cost of the total exercise intervention and the net energy cost of the exercise of the final week of the intervention (Table 5.2).

Separate analysis of the Control group did not show significant correlations between the exercise-mediated change in insulin sensitivity and energy expended during the exercise intervention (Table 5.2). However, a reduction in both leptin and $\text{TNF}\alpha$ was significantly correlated with improved insulin sensitivity whilst increased fasting 3-OHB concentration was associated with improved insulin sensitivity (Table 5.2, Figure 5.3). A reduction in energy intake was also associated with improved insulin sensitivity in Controls (Table 5.2).

In Offspring, improved insulin sensitivity was positively correlated with net energy expended during the complete intervention and during the final week of exercise, but was not associated with the net energy cost of the final session (Table 5.2). In addition, reduced lower body fat, circulating CRP and leptin were also associated with improved insulin sensitivity in those with a family history of T2D (Table 5.2, Figure 5.3). Reduction in resting heart rate and increased habitual physical activity after the intervention was associated with improved insulin sensitivity (Table 5.2).

Figure 5.3 Scattergram showing relationship between the change in ln leptin and the change in ln ISI.



Insulin sensitivity at baseline was strongly associated with adiposity in both groups. Therefore, in view of an apparent relationship between circulating leptin and both baseline insulin sensitivity, and exercise-mediated change in insulin sensitivity, correlations were performed between all measured variables and residuals of the regression of the change in ln ISI against the change in total fat mass (Figure 5.4). After correcting for fat mass in this manner, reductions in circulating leptin concentration, lower body fat mass, resting heart rate and an increase in habitual physical activity remained correlated with the change in insulin sensitivity in the combined group (Table 5.2). When analysed separately, the Controls exhibited a significant positive correlation between fasting 3-OHB concentration and change in insulin sensitivity (Table 5.2). The exercise-mediated change in insulin sensitivity in the Offspring group was associated with a reduction in lower body fat mass, hip circumference, resting heart rate and both circulating leptin and CRP concentrations (Table 5.2). Offspring also showed a significant positive correlation between fat mass adjusted change in insulin

sensitivity and the surrogate marker of habitual physical activity, average heart rate minus resting heart rate (Table 5.2).

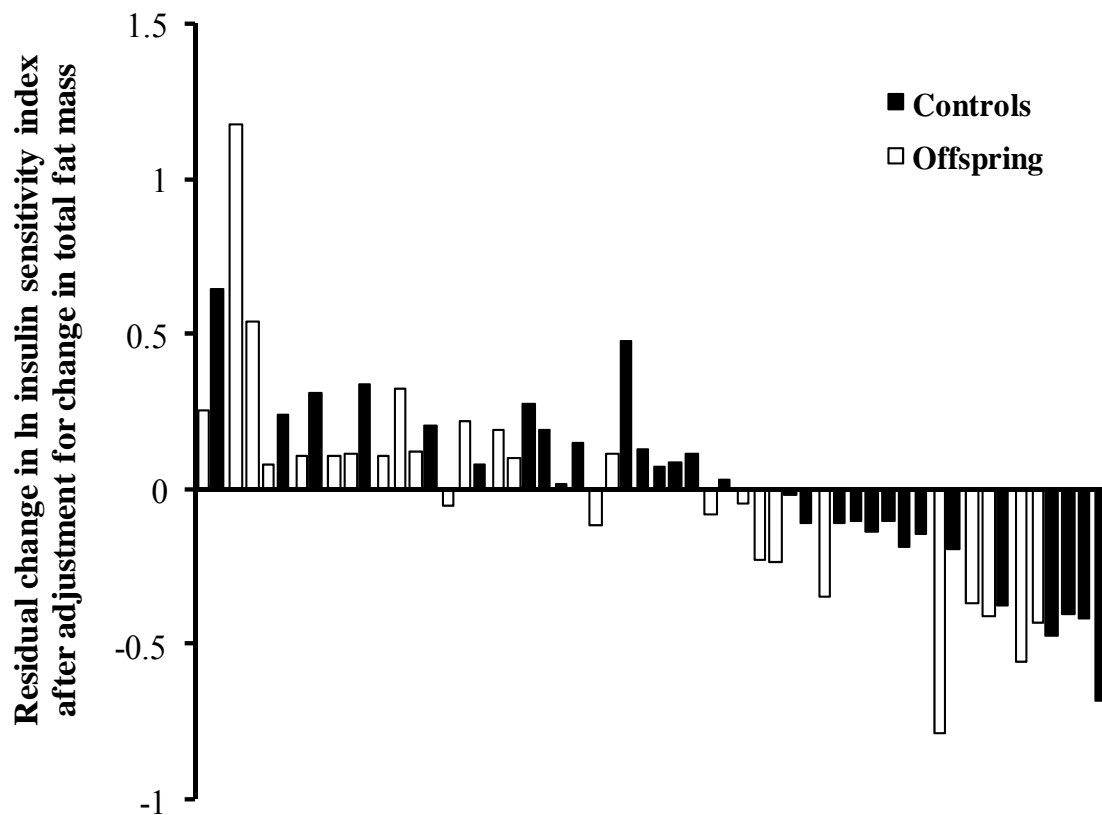


Figure 5.4 Individual changes in ln insulin sensitivity index (ISI) residuals after adjustment for change in fat mass in whole group ($n = 61$) after exercise intervention. Volunteers ranked in order of change in unadjusted ISI.

After adjustment for the change in fat mass, baseline insulin sensitivity continued to influence the change in insulin sensitivity after exercise in the combined group ($r = -0.43$, $p < 0.01$), Control group ($r = -0.41$, $p < 0.05$) and Offspring group ($r = -0.49$, $p < 0.05$). Due to the apparent influence of baseline insulin sensitivity on the exercise mediated change in insulin sensitivity, correlations were performed between all measured variables and residuals for the regression of the change in ln ISI against baseline ln ISI (Figure 5.5). After correction for baseline insulin sensitivity, only the reductions in circulating leptin concentration and lower

body fat mass and increase in habitual physical activity were still significantly associated with the change in insulin sensitivity after the exercise intervention in the combined group (Table 5.2). In the Control group, after adjustment for baseline insulin sensitivity; energy intake, circulating leptin and TNF α concentrations were all negatively correlated with post-intervention change in insulin sensitivity and change in fasting 3-OHB was positively correlated with improved insulin sensitivity (Table 5.2). In the Offspring group only reduced circulating leptin concentration remained significantly correlated with the improved insulin sensitivity after adjustment for baseline ISI (Table 5.2).

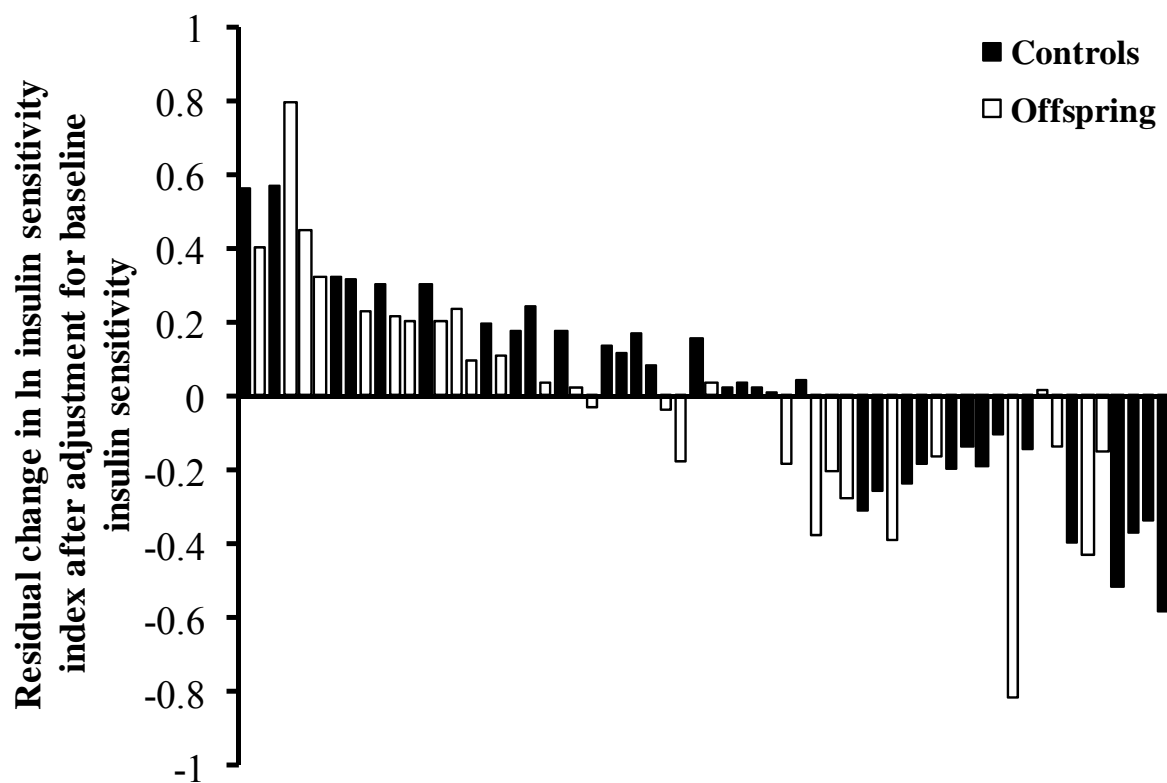


Figure 5.5 Individual changes in ln insulin sensitivity index (ISI) residuals after adjustment for baseline insulin sensitivity in whole group (n = 62) after exercise intervention. Volunteers ranked in order of change in unadjusted ISI.

Table 5.2 Correlations between post-exercise variable change and change in ln ISI unadjusted (a) and adjusted for: (b) fat mass, (c) baseline ISI.

Correlations with change in insulin sensitivity										
		Combined			Controls			Offspring		
		(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)
Intervention variables	Total net energy expenditure of exercise	0.33 (< 0.05)	-	-	-0.32 (0.12)	-	-	0.74 (< 0.0001)	-	-
	Final week net exercise energy expenditure	0.33 (< 0.05)	-	-	-0.38 (0.07)	-	-	0.65 (< 0.001)	-	-
	Last session net energy expenditure	0.19 (0.20)	-	-	-0.14 (0.50)	-	-	0.37 (0.08)	-	-
Body composition variables	Change in fat mass	-0.11 (0.42)	-	0.06 (0.65)	-0.04 (0.67)	-	-0.16 (0.41)	-0.05 (0.80)	-	0.25 (0.21)
	Change in lower body fat mass ^a	-0.35 (< 0.01)	-0.36 (< 0.01)	-0.29 (< 0.05)	-0.16 (0.39)	-0.14 (0.45)	-0.24 (0.20)	-0.46 (< 0.05)	-0.49 (< 0.05)	-0.31 (0.11)
	Change in hip circumference ^a	-0.29 (< 0.05)	-0.24 (< 0.08)	-0.23 (0.08)	-0.09 (0.63)	0.08 (0.66)	-0.17 (0.35)	-0.48 (< 0.05)	-0.45 (< 0.05)	-0.29 (0.14)
Physiological/ metabolic variables	Change in $\dot{V}O_{2\max}$	-0.10 (0.42)	-0.10 (0.47)	-0.05 (0.72)	-0.24 (0.17)	-0.19 (0.32)	-0.26 (0.14)	-0.06 (0.78)	-0.04 (0.85)	0.13 (0.51)
	Change in RMR	0.08 (0.52)	0.11 (0.45)	-0.03 (0.81)	-0.06 (0.76)	-0.12 (0.53)	-0.15 (0.40)	0.15 (0.46)	0.23 (0.28)	0.05 (0.82)
	Change in Post-glucose MR	-0.12 (0.37)	-0.15 (0.28)	-0.15 (0.24)	-0.26 (0.13)	-0.34 (0.07)	-0.25 (0.15)	0.11 (0.60)	0.09 (0.71)	-0.01 (0.97)

		Correlations with change in insulin sensitivity								
		Combined			Control			Offspring		
		(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)
Behavioural variables	Change in Energy intake	-0.09	0.00	-0.10	-0.43	-0.30	-0.41	0.11	0.14	0.11
		(0.53)	(0.99)	(0.48)	(< 0.05)	(0.15)	(< 0.05)	(0.60)	(0.50)	(0.59)
	Change in Resting HR	-0.27	-0.34	-0.20	-0.23	-0.20	-0.22	-0.41	-0.48	-0.19
		(< 0.05)	(< 0.05)	(0.13)	(0.22)	(0.33)	(0.24)	(< 0.05)	(< 0.05)	(0.33)
	Change in Average HR – Resting HR	0.41	0.35	0.31	0.21	0.20	0.25	0.50	0.48	0.35
		(< 0.01)	(< 0.01)	(< 0.05)	(0.30)	(0.33)	(0.22)	(< 0.01)	(< 0.05)	(0.07)
Biochemical variables	Change in leptin ^a	-0.43	-0.32	-0.40	-0.35	-0.31	-0.44	-0.40	-0.39	-0.42
		(< 0.0005)	(< 0.05)	(< 0.01)	(< 0.05)	(0.10)	(< 0.05)	(< 0.05)	(< 0.05)	(< 0.05)
	Change in CRP ^a	-0.05	-0.16	-0.01	0.18	0.10	0.18	-0.53	-0.51	-0.30
		(0.70)	(0.25)	(0.94)	(0.31)	(0.61)	(0.32)	(< 0.01)	(< 0.01)	(0.12)
	Change in TNFα ^a	-0.09	-0.09	-0.13	-0.35	-0.34	-0.38	0.14	0.13	0.13
		(0.47)	(0.50)	(0.30)	(< 0.05)	(0.06)	(< 0.05)	(0.47)	(0.52)	(0.50)
	Change in Fasting 3-OHB ^a	0.13	0.20	0.24	0.41	0.40	0.38	-0.06	0.02	0.09
		(0.92)	(0.13)	(0.06)	(< 0.05)	(< 0.05)	(< 0.05)	(0.94)	(0.91)	(0.67)

n = 62, values are correlation coefficients with p-values in brackets. (a): unadjusted data; (b): variables correlated against ln ISI adjusted for change in fat mass; (c): variables correlated against ln ISI adjusted for baseline ln ISI. ^aStatistical analysis performed on logarithmically transformed data. Significant correlations in bold.

The exercise-mediated change in insulin sensitivity was significantly correlated with the net energy cost of the total exercise intervention and the net energy cost of the exercise performed in the final week of the study. In order to determine which variables might influence exercise-mediated alterations in insulin sensitivity, correlations were performed between all measured variables and residuals for the regression of the change in ln ISI against firstly: the net energy cost of the total exercise intervention and secondly: the net energy cost of the exercise in the final week of the intervention (Figure 5.6). The energy cost of the final session of the intervention was not associated with the change in insulin sensitivity in either the combined, Control or Offspring groups.

In the combined group, changes in lower body fat, resting heart rate and circulating leptin were negatively correlated, and changes in habitual physical activity positively correlated, with the exercise-mediated change in insulin sensitivity after adjustment for net total exercise energy expenditure (Table 5.3). After adjustment for the energy cost of the final week the previously observed association with change in resting heart rate was no longer statistically significant (Table 5.3). After adjustment for the net energy cost of the total exercise intervention, the Control group displayed significant negative correlations between the changes in energy intake, circulating leptin and TNF α and the change in insulin sensitivity (Table 5.3). A positive correlation between the change in fasting 3-OHB concentration and the change in insulin sensitivity was observed after adjustment for the net energy expended during the final week of the intervention (Table 5.3). In the Offspring group adjustment for both the net total energy expenditure and the net energy expenditure of the final week of the intervention did not alter the significant correlations observed in unadjusted regressions, except for the association between the change in leptin concentrations and the change in insulin sensitivity, which was no longer significant (Table 5.2, 5.3).

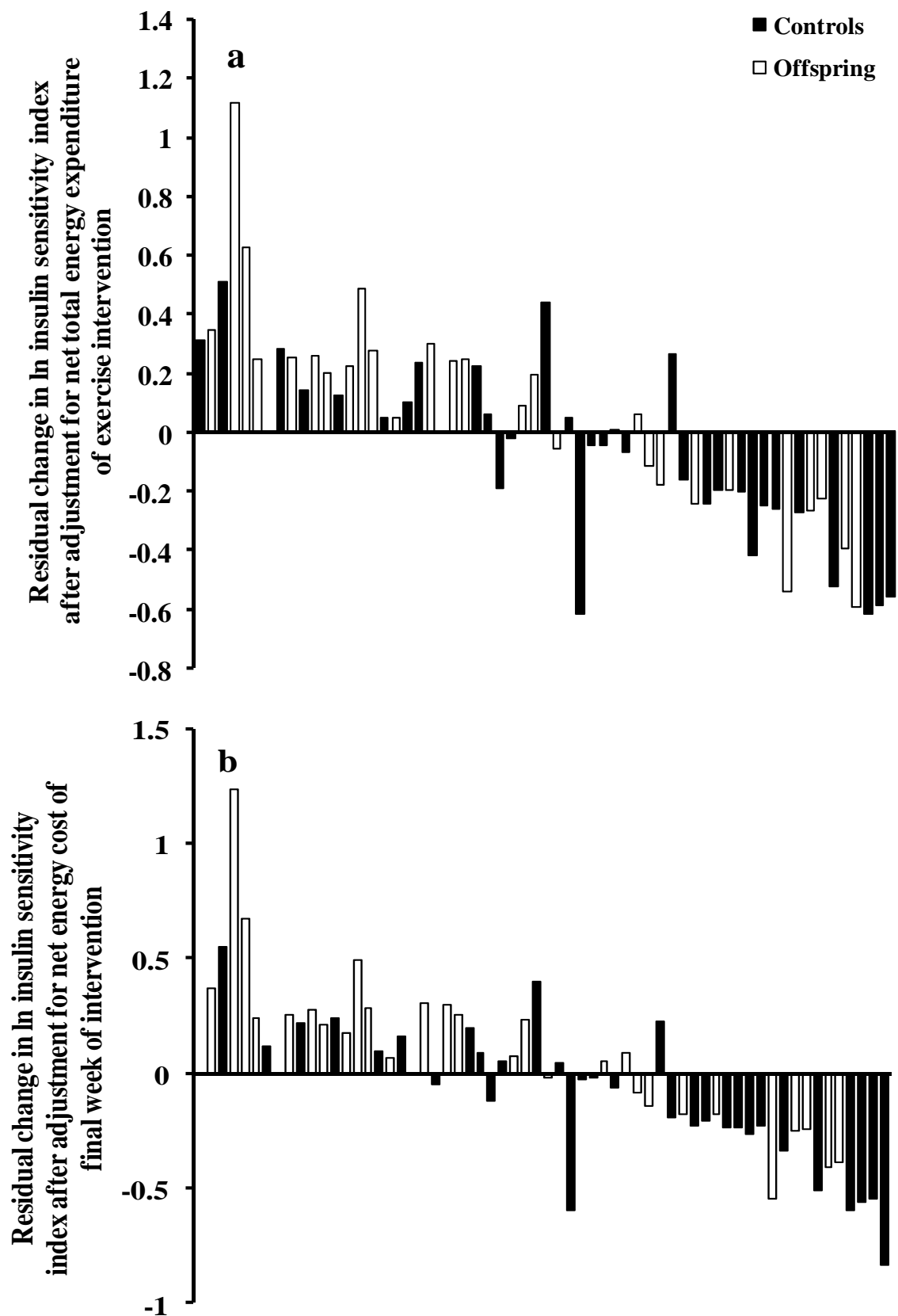


Figure 5.6 Individual changes in ln ISI residuals after adjustment for (a) net total energy expenditure of exercise, (b) net total energy expenditure of final week of exercise in whole group (n = 60). Volunteers ranked in order of change in unadjusted ISI.

Table 5.3 Correlations between post-exercise variable change and ln ISI adjusted for (d) net total energy expenditure of the exercise intervention, (e) net total energy expenditure of final week of exercise, (f) net energy expenditure of final exercise session.

Correlations with change in insulin sensitivity										
		Combined			Controls			Offspring		
		(d)	(e)	(f)	(d)	(e)	(f)	(d)	(e)	(f)
Body composition variables	Change in fat mass	-0.11	-0.10	-0.05	-0.01	0.04	-0.03	-0.08	-0.06	-0.05
		(0.42)	(0.48)	(0.73)	(0.95)	(0.98)	(0.88)	(0.69)	(0.78)	(0.80)
	Change in lower body fat mass ^a	-0.28	-0.32	-0.35	-0.09	-0.14	-0.16	-0.38	-0.44	-0.46
		(< 0.05)	(< 0.05)	(< 0.01)	(0.67)	(0.46)	(0.40)	(< 0.05)	(< 0.05)	(< 0.05)
	Change in hip circumference ^a	-0.23	-0.21	-0.25	-0.05	0.15	0.12	-0.39	-0.45	-0.47
	(0.08)	(0.11)	(0.06)	(0.79)	(0.43)	(0.53)	(< 0.05)	(< 0.05)	(< 0.05)	
Physiological/ metabolic variables	Change in $\dot{V}O_{2\max}$	-0.17	-0.09	-0.07	-0.32	-0.16	-0.16	-0.11	-0.09	-0.06
		(0.20)	(0.49)	(0.58)	(0.08)	(0.40)	(0.41)	(0.58)	(0.65)	(0.76)
	Change in RMR	0.06	0.09	0.09	-0.06	-0.02	-0.03	0.12	0.14	0.15
		(0.65)	(0.50)	(0.49)	(0.74)	(0.90)	(0.87)	(0.54)	(0.47)	(0.47)
	Change in Post-glucose MR	-0.14	-0.11	-0.12	-0.27	-0.21	-0.25	0.07	0.08	0.10
	(0.30)	(0.42)	(0.39)	(0.14)	(0.25)	(0.17)	(0.73)	(0.68)	(0.62)	

		Correlations with change in insulin sensitivity								
		Combined			Control			Offspring		
		(d)	(e)	(f)	(d)	(e)	(f)	(d)	(e)	(f)
Behavioural variables	Change in Energy intake	-0.06	-0.05	-0.05	-0.49	-0.33	-0.33	-0.20	0.12	0.11
		(0.70)	(0.73)	(0.73)	(< 0.05)	(0.11)	(0.10)	(0.30)	(0.56)	(0.60)
	Change in Resting HR	-0.27	-0.26	-0.27	-0.20	-0.22	-0.22	-0.42	-0.40	-0.42
		(< 0.05)	(0.06)	(< 0.05)	(0.29)	(0.27)	(0.27)	(< 0.05)	(< 0.05)	(< 0.05)
	Change in Average HR – Resting HR	0.37	0.39	0.41	0.25	0.21	0.21	0.40	0.46	0.49
		(< 0.01)	(< 0.01)	(< 0.01)	(0.24)	(0.30)	(0.30)	(< 0.05)	(< 0.05)	(< 0.01)
Biochemical variables	Change in leptin ^a	-0.38	-0.41	-0.44	-0.36	-0.27	-0.30	-0.30	-0.36	-0.39
		(< 0.01)	(< 0.01)	(< 0.01)	(< 0.05)	(0.15)	(0.10)	(0.12)	(0.06)	(< 0.05)
	Change in CRP ^a	-0.07	-0.07	-0.08	0.16	0.11	0.10	-0.53	-0.53	-0.53
		(0.62)	(0.59)	(0.54)	(0.39)	(0.56)	(0.58)	(< 0.01)	(< 0.01)	(< 0.01)
	Change in TNFα ^a	-0.16	-0.08	-0.08	-0.52	-0.32	-0.32	0.16	0.15	0.14
		(0.23)	(0.54)	(0.57)	(< 0.01)	(0.08)	(0.08)	(0.43)	(0.46)	(0.47)
		0.06	0.21	0.20	0.22	0.42	0.41	-0.05	0.01	0.02
		(0.63)	(0.12)	(0.13)	(0.23)	(< 0.05)	(< 0.05)	(0.79)	(0.98)	(0.94)

n = 62, values are correlation coefficients with p-values in brackets. (d): ISI adjusted for total net energy expenditure of intervention; (e): ISI adjusted for total net energy expenditure of final week of intervention; (f): ISI adjusted for total net energy expenditure of final session of intervention. ^aStatistical analysis performed on logarithmically transformed data. Significant correlations in bold.

5.4.4 Multivariate analysis

To determine whether the greater improvement in insulin sensitivity in the Offspring in response to the exercise intervention could be explained by differences in changes in fitness, energy intake, body fat, compliance to the exercise intervention or energy expended during the intervention between the Control and Offspring groups, multivariate regression analyses were undertaken. Change in insulin sensitivity was the response variable and group (Control or Offspring), together with number of minutes of exercise completed, energy expended during the intervention, change in body fat, change in energy intake, change in $\dot{V}O_{2\max}$ or change in $\dot{V}O_2$ at lactate threshold, as the predictor variables. In all of these analyses, group remained a significant predictor of the change in insulin sensitivity in response to the intervention (all $p < 0.05$), whereas none of the other factors included in the models were significant predictors of the change in insulin sensitivity. Thus, the finding that insulin sensitivity increased to a greater extent in the Offspring than the Control group was not confounded by differences in compliance to the exercise intervention, energy expended during exercise, differences in fat loss or differences in changes in fitness between the groups.

When change in leptin concentration and group were included in a multivariate regression model, change in leptin was a significant predictor of change in insulin sensitivity ($p < 0.01$), whereas the statistical significance of group was lost ($p = 0.29$), indicating that the greater change in insulin sensitivity in the Offspring group was associated with their greater change in circulating leptin. When change in fat mass was added to this model, change in leptin remained a significant predictor of change in insulin sensitivity ($p < 0.0005$). Additionally, in multivariate regression analysis including baseline insulin sensitivity and group as the predictor variables, baseline insulin sensitivity was a stronger significant predictor of the change in insulin sensitivity ($p < 0.0005$) than group ($p = 0.13$) (Figure 5.3). When baseline insulin sensitivity, change in leptin and group were included in the same model, baseline insulin sensitivity ($p < 0.0005$) and change in leptin ($p < 0.0005$) were both independent

predictors of change in insulin sensitivity, whereas the significant effect of group was lost ($p = 0.81$). In view of the correlations observed in section 5.3, the change in CRP, adiponectin, TNF α and 3-OHB were added to the model, however only the change in leptin ($p < 0.0005$) and baseline insulin sensitivity ($p < 0.0005$) remained independent predictors of the change in insulin sensitivity. The addition of behavioural variables such as the change in energy intake, resting heart rate and habitual physical activity to the model determined similar results for the change in leptin and baseline insulin sensitivity but added the change in energy intake ($p < 0.05$) as a significant independent predictor of exercise-mediated change in insulin sensitivity. This model, which included all factors with significant univariate correlations, explained 47.9% of the variance in the change in insulin sensitivity in response to exercise (adjusted R^2).

5.5 Discussion

The main findings of this study were that improved exercise-mediated insulin sensitivity was observed in those who were most insulin resistant at baseline. This differs slightly from the results in Chapter 4 where Offspring displayed the greatest improvement in insulin sensitivity therefore baseline insulin sensitivity is a stronger predictor of exercise response than a family history of diabetes. Additionally, the insulin sensitivity response was greatest in those who reduced circulating leptin concentrations, resting heart rate and lower limb fat mass and those who increased habitual physical activity and expended the most energy during the intervention. Despite the strong associations between baseline adiposity and insulin sensitivity, the associations between the exercise-mediated increase in insulin sensitivity and leptin concentration, baseline insulin sensitivity, lower limb fat mass and habitual physical activity were independent of changes in fat mass. Reductions in circulating leptin, lower body fat mass and increased habitual physical activity continued to positively influence insulin sensitivity after adjustment for the influence of baseline insulin sensitivity. After adjustment for the net energy cost of the exercise intervention, reduction in lower body fat mass, circulating leptin and an increase in habitual physical activity remained associated with improved insulin sensitivity after exercise.

Exercise-mediated changes in anthropometric variables were associated with improved insulin sensitivity in response to the exercise programme. A reduction in lower body fat mass was associated with improved post-exercise insulin sensitivity in the combined and Offspring groups and was independent of reduced total fat mass, energy expended during exercise and in the combined group, baseline insulin sensitivity. The observation of a reduction in lower limb fat mass appearing to influence insulin sensitivity is surprising, since reductions in visceral and total abdominal adipose tissue are more frequently associated with this outcome (Boudou et al. 2003; O'Leary et al. 2006). However, this association has previously been reported in a smaller study of obese subjects (Goodpaster et al. 2003), and it has been

suggested that thigh fat may be a marker of intramuscular or deep subcutaneous fat accumulation, which is recognised to correlate with insulin sensitivity (Goodpaster, Thaete, & Kelley 2000; Ryan, Nicklas, & Berman 2002; Yim et al. 2007). In the Offspring group, reduced lower body fat did not correlate with insulin sensitivity after adjustment for baseline insulin sensitivity and was not a significant independent predictor of exercise-mediated increased insulin sensitivity in multivariate analysis. The participants in this study engaged in a variety of exercise activities, however although the majority of these were lower limb dominant (walking, running, cycling) there is no evidence that this preferentially favours lower limb fat reduction (McTiernan et al. 2007; Nindl et al. 2000; Wilmore et al. 1999). It is therefore unclear why reduced lower body fat was correlated with improved insulin sensitivity, and why this observation was not significant in Controls. More detailed imaging of adipose tissue compartments, direct assessment of intramuscular lipid content or greater knowledge of the specific metabolic role of deep subcutaneous adipose tissue (Sniderman et al. 2007) would be required to provide evidence which would agree with the observations of Goodpaster and colleagues (Goodpaster, Thaete, & Kelley 2000; Goodpaster et al. 2003).

Lifestyle factors were associated with increased insulin sensitivity in response to exercise. The surrogate index of habitual physical activity, average heart rate minus resting heart rate, was associated with increased insulin sensitivity in the combined and Offspring, but not the Control groups. Increased habitual physical activity was associated with improved insulin sensitivity in the combined group independent of change in fat mass, baseline insulin sensitivity and energy expended during exercise. Increased habitual physical activity was correlated with improved insulin sensitivity in Offspring independent of change in fat mass and energy expenditure of exercise but not baseline insulin sensitivity. Offspring showed a significant increase in habitual physical activity during the intervention and the observation that this factor was not independent of baseline insulin sensitivity may suggest that the more insulin resistant volunteers were those who increased physical activity most.

A reduction in resting heart rate was also correlated with increased insulin sensitivity in the combined and Offspring groups, and was independent of change in fat mass and the energy expenditure of exercise but not of baseline insulin sensitivity. Reduced resting heart rate can be considered to be a crude marker of improved cardiorespiratory fitness (Jurca et al. 2005), however a more accurate description of the change in cardiorespiratory fitness, the change in predicted $\dot{V}O_{2max}$, was not associated with increased insulin sensitivity in any of the groups. High resting heart rate may be indicative of dysfunction of the autonomic nervous system which in turn is recognised to influence inflammatory responses and glucose metabolism (Carnethon & Craft 2008; Tracey 2002). Chronic sympathetic over-stimulation may promote an inflammatory and pro-glycaemic state which contributes to insulin resistance and endothelial dysfunction (Carnethon et al. 2003; Piwonska et al. 2008; Thayer & Fischer 2009). Sympathetic hyperactivity has been observed in offspring of subjects with T2D and correlates with insulin resistance (Huggett et al. 2006). It is unclear whether sympathetic hyperactivity is a cause, or an effect, of insulin resistance (Huggett et al. 2006); however sympathetic hyperactivity could theoretically augment insulin resistance by stimulation of chronic inflammation (Egan 2003). Therefore, reduction in resting heart rate in response to exercise may represent reversal of early autonomic dysfunction which is associated with insulin resistance and a mechanism by which exercise might improve insulin sensitivity (Giallauria et al. 2008b). In this study, reduced resting heart rate was associated with improved insulin sensitivity in Offspring and combined groups but was not independent of baseline insulin sensitivity. This may suggest that those with greater baseline insulin resistance also have a degree of early autonomic dysfunction which contributes to the aetiology of the insulin resistance but which is sensitive to the effects of exercise (Frontoni et al. 2003; Giallauria et al. 2008).

Baseline resting and post-glucose metabolic rates were correlated with an exercise-mediated increase in insulin sensitivity. Higher resting metabolic rates have been recorded in obese compared to lean women (Weststrate et al. 1990), therefore it might have been expected to

observe a correlation between reductions in metabolic rate and improved insulin sensitivity which was dependent upon change in fat mass. However, no change in metabolic rate or substrate utilisation in response to the exercise intervention was observed to significantly correlate with the change in insulin sensitivity. This is a surprising finding, particularly in the Offspring group who manifest significantly increased fat oxidation and reduced carbohydrate oxidation after the intervention (Section 4.4.3.8). Furthermore, there is consistent evidence to support an association between increased fat oxidation and insulin sensitivity in other studies (Gan et al. 2003; Goodpaster et al. 2003). However, these associations were reported in obese subjects and have not been observed in relatives of patients with type 2 diabetes (Ostergard et al. 2006). Although increases in fat oxidation are recognised in response to an exercise intervention (Blaak & Saris 2002), these changes may not influence insulin sensitivity directly, given that a weight loss intervention has been shown to increase insulin sensitivity to the same degree as a weight loss plus exercise intervention, but with increased oxidative capacity observed in the exercise group alone (Schenk et al. 2009). Additionally, Schenk and colleagues (2009) have shown that despite increased fat oxidation in the exercise plus weight loss group, lipid infusion can reverse the improved insulin sensitivity to the same extent in both groups (Schenk et al. 2009). It has also been previously suggested that whole body indirect calorimetry may be a relatively crude method of assessing alterations in skeletal muscle fat oxidation (Galgani et al. 2008; Galgani, Moro & Ravussin 2008). Although exercise has been shown to increase fat oxidation in association with reduced circulating leptin and reduced intramuscular lipid content it has not been associated with the observed increase in insulin sensitivity (Solomon et al. 2008).

Improved insulin sensitivity in Controls alone was correlated with an increase in fasting hepatic fatty acid oxidation (as assessed by 3-OHB concentrations), and was independent of both baseline insulin sensitivity and change in fat mass. However, this association was not independent of the net energy cost of the exercise intervention. Exercise is recognised to increase hepatic fat oxidation (Gill et al. 2007), reduce hepatic steatosis and increase total

body insulin sensitivity (Shah et al. 2009b). However, a six-week aerobic exercise intervention has been associated with improved hepatic insulin sensitivity (characterised by reduced endogenous glucose production in response to insulin infusion) without evidence of reduced steatosis (Shojaee-Moradie et al. 2007). Therefore a mechanism in which exercise stimulates oxidation of intrahepatic lipid directly resulting in increased insulin sensitivity would appear to be an oversimplification (Johanson et al. 2003). Black and colleagues (2005) observed improved hepatic insulin sensitivity after exercise only in the context of energy deficit however measurements of total body and hepatic fatty acid metabolism were not performed (Black et al. 2005). Neither Controls nor Offspring displayed a significant increase in fasting hepatic fat oxidation after the exercise intervention (Section 4.4.3.2) and the absence of a significant independent contribution to the exercise mediated change in insulin sensitivity in multivariate analysis suggests that increased fasting hepatic fatty acid oxidation is not a key determinant of the exercise-mediated change in insulin sensitivity.

Reductions in circulating TNF α were correlated with increased insulin sensitivity in the Control group, however these associations were not observed after adjustment for the change in fat mass or the net energy cost of the final week and final session of the intervention. Exercise intervention in obese women has been shown to reduce circulating TNF α concentrations (Kondo, Kobayashi, & Murakami 2006), and a combined diet and physical activity intervention in obese women has shown a significant association between the amount of weight lost and the reduction in circulating TNF α (Zahorska-Markiewicz et al. 2008). The results from the current study would be consistent with the previous observations that exercise-mediated reductions in circulating TNF α are more strongly associated with fat mass than with insulin sensitivity (Polak et al. 2006). Additionally, it has been suggested that TNF α influences insulin sensitivity in a paracrine fashion and that circulating TNF α may not be the best marker of the influence of changes in the TNF system on insulin sensitivity (Strackowski et al. 2001). Thus, the observed association between reduced TNF α and insulin

sensitivity in Controls is likely to be related to reduced fat mass, rather than a direct effect on insulin sensitivity and this suggestion is supported by the multivariate regression model.

Reduced CRP was significantly correlated with improved insulin sensitivity in the Offspring group but not the combined or Control groups. The association was independent of the net energy cost of the intervention and the influence of reduced fat mass, but was not significant after adjustment for baseline insulin resistance. Dekker and colleagues (2007) observed greater CRP concentrations in obese subjects, compared to lean subjects and subjects with T2D (Dekker et al. 2007). Changes in insulin sensitivity in response to exercise were not associated with change in CRP in this study, but it must be appreciated that Dekker and co-workers analysed the effects of an exercise intervention with preserved body mass and that this study had only 8 subjects in each group and was under-powered to detect correlations between insulin sensitivity and other variables which did not change over the intervention (Dekker et al. 2007). Balducci and colleagues (2009) described reductions in CRP after a twelve month exercise intervention in patients with type 2 diabetes (Balducci et al. 2009). Balducci and co-workers observed greater reduction in CRP and other indices of inflammation and increased insulin sensitivity in those who participated in high intensity aerobic activity, and a maximal response in those who engaged in a mixed aerobic and resistance regime (Balducci et al. 2009). However, there was no reported correlation between the reduction in CRP and the increase in insulin sensitivity and the methods of assessing insulin sensitivity (HOMA-IR) in subjects with confirmed T2D who are treated with a range of pharmaceuticals can be questioned (Anderson et al. 1995). A number of other authors have found that exercise-mediated improvements in insulin sensitivity are unrelated to changes in CRP (Marcell et al. 2005; Oberbach et al. 2006), whilst Kadoglou and colleagues (2007) reported a significant reduction in CRP after exercise in patients with T2D, but did not report whether this was associated with improved insulin sensitivity (Kadoglou et al. 2007). The results from the current study would suggest that CRP is a marker, rather than a mediator, of insulin resistance and that it is indicative of a potential beneficial effect of exercise only in

that it identifies those who are more insulin resistant. This supposition would be supported by the absence of change in CRP as a significant independent predictor of the exercise-mediated change in insulin sensitivity in multivariate analysis.

Reduced circulating leptin was significantly correlated with improved insulin sensitivity in the combined, Control and Offspring groups. However the association was dependent upon adiposity and the net energy expended during the final week and final session of the intervention in the Control group. In Control subjects the influence of reduced leptin appears to be inter-related to reduced fat mass, it is therefore difficult to be certain whether reduction in leptin exerts an insulin sensitising effect or whether it is simply a marker of adiposity. The relationship between leptin and insulin sensitivity was independent of the energy cost of the intervention, but not the energy cost of either the final week or the final session. This would suggest that in Controls, the effect of leptin on insulin sensitivity is related to reduced fat mass and an acute effect of exercise (Olive & Miller 2001). For Controls, one unifying pathway which links the associations between changes in energy intake, fasting hepatic fatty acid oxidation, leptin concentration and the exercise-mediated change in insulin sensitivity is through stimulation of AMP-kinase (AMPK). Leptin is recognised to act centrally (via AMPK) on the hypothalamus to reduce appetite and to stimulate fat oxidation in hepatic and skeletal muscle cells (Hutchinson, Summers, & Bengtsson 2008). However, no change in total body fat oxidation was observed in Controls therefore the suggestion that improved leptin sensitivity might mediate improved insulin sensitivity through increased fat oxidation cannot be substantiated by these results.

In Offspring, the influence of reduced leptin on insulin sensitivity was independent of the change in fat mass and the degree of baseline insulin resistance, but not the net energy cost of the total or final week of the intervention. This may reflect a mechanism by which energy deficit, independent of fat loss leads to reduction in circulating leptin. The relationship

between exercise-mediated energy deficit and reduced leptin concentration has previously been described (McMurray & Hackney 2005). Minokoshi and colleagues (2002) have identified increased skeletal muscle fat oxidation triggered by leptin-stimulated, AMPK-mediated suppression of acetyl CoA carboxylase (Minokoshi et al. 2002). In addition, previously observed increases in insulin sensitivity and reductions in leptin are thought to be related to the impact of exercise on fat oxidation, rather than the change in fat mass (Goodpaster et al. 2003; Solomon et al. 2008). However despite significantly increased fat oxidation in Offspring, the exercise-mediated improvement in insulin sensitivity in this group was not correlated with fat oxidation. Therefore, despite a plausible metabolic pathway by which reduced leptin concentrations reflect improved leptin sensitivity which in turn mediates improved fat oxidation and subsequently increased insulin sensitivity, the results of this chapter do not support this supposition.

Despite a positive correlation between adiponectin and insulin sensitivity at baseline, there were no associations between the change in adiponectin and the change in insulin sensitivity after the exercise intervention. Positive correlations between improvement in insulin resistance and adiponectin have previously been observed (Bluher et al. 2006; Oberbach 2006). However, Oberbach and colleagues only observed this association in subjects with impaired glucose tolerance (IGT) or type 2 diabetes (Oberbach et al. 2006) and although Bluher and co-workers did observe a change in subjects with normal glucose tolerance, this was in an older population than the current study. Monzillo and colleagues also illustrated improved insulin sensitivity correlated with increased adiponectin, however this was only found in patients with type 2 diabetes, and in response to a combined physical activity and hypocaloric diet regime (Monzillo et al. 2003). This is consistent with the work of Hotta and colleagues (2000), who observed increased adiponectin after dietary induced weight loss (Hotta et al. 2000). Other recent studies have observed exercise mediated changes in insulin sensitivity in subjects with type 2 diabetes (Boudou et al. 2003), obese women (Polak et al. 2006), and subjects with IGT which are not associated with changes in circulating

adiponectin. Additionally, Polak and colleagues (2007) reported an increase in total and multimeric adiponectin but no relationship between adiponectin and insulin sensitivity at baseline or after dietary-induced weight loss (Polak et al. 2007). There may be a number of reasons why adiponectin is not significantly associated with exercise mediated change in insulin sensitivity in the current study. Firstly, the volunteers in the current study were normally glucose tolerant, and therefore compared to subject groups who did show an association between exercise mediated change in insulin sensitivity and an increase in plasma adiponectin, the current volunteers may be too insulin sensitive to trigger an exercise mediated response in adiponectin (Oberbach et al. 2006). Additionally, if adiponectin acts to stimulate hepatic fat oxidation as a compensatory response to increased circulating NEFA (Bonet et al. 2007), then increased lipid oxidation in muscle in response to improved leptin sensitivity (Solomon et al. 2008) may downregulate adiponectin, particularly since adiponectin may increase skeletal muscle lipid oxidation in a similar AMPK dependent pathway to both leptin and exercise (Boudou et al. 2003; Yamauchi et al. 2002). Finally, adiponectin has recently been discovered to have more than one active form, and therefore the assay used in this (and other) studies may not have detected changes in different types of adiponectin, which may have differing metabolic properties (Yamauchi & Kadowaki 2008).

Multivariate regression analysis produced a model in which parental history of diabetes was a significant predictor of exercise mediated improvement in insulin sensitivity. However, any individual, regardless of family history, who displays insulin resistance prior to exercise, or who displays an ability to reduce circulating leptin (independent of fat mass) would be expected to improve insulin sensitivity after an exercise intervention to a greater degree than a matched individual who is more insulin sensitive or who does not modulate leptin in response to exercise. This observation is consistent with previous evidence which found serum leptin to be a stronger predictor of insulin resistance than diabetes family history (Nyholm et al. 1997). Reduction in circulating leptin also predicted improvements in insulin resistance in individuals with IGT who participated in a diet and exercise intervention

(Corpeleijn et al. 2007). To date, this study is the first to describe improved insulin sensitivity, mediated by reduced circulating leptin in response to exercise, in relatives of patients with type 2 diabetes.

This study has a number of limitations, the imaging modality utilised to assess body composition was unable to delineate separate subcutaneous adipose compartments, or determine intramuscular lipid content, both of which would have been of value in examining the anthropometric mediators of exercise-mediated change in insulin sensitivity. In addition, investigation of fat oxidation was limited to whole body assessment by indirect calorimetry and may not be sensitive enough to determine specific alterations in skeletal muscle oxidative function, however this method was sensitive enough to determine a different post-intervention effect in Controls and Offspring. This study did not assay high molecular weight adiponectin, or circulating TNF α receptor concentrations and therefore lacks the ability to more completely determine adipose hormone function. Further exploration of adipose tissue hormones and exercise is warranted to determine the interaction between change in fat mass, energy deficit and insulin sensitivity. The analysis contained within this chapter was an attempt to determine whether exercise-mediated alterations in other variables were significantly associated with the change in insulin sensitivity. For this reason, the results and conclusions contained within this chapter cannot be considered to be definitive evidence of a mechanistic relationship. Instead, these associations suggest possible mechanisms allowing the generation of hypotheses which relate exercise to alterations in metabolic health and are therefore intended to provide a basis for future hypothesis driven research. Although it may be argued that a more focused approach to the mechanisms which mediate the insulin-sensitising response might have been appropriate, the complexity of studying a large group of free living individuals should be appreciated therefore inclusion of variables such as diet and habitual activity were felt to be important to prevent confounding. In addition, whilst the inclusion of further variables in the analysis may reduce the statistical power of the conclusions it was preferable to minimise the statistical significance of any interaction than to

ignore a potential confounding factor which would obscure associations which might guide further study.

In summary, exercise-mediated increase in insulin sensitivity can be predicted by a positive family history of type 2 diabetes, although baseline insulin resistance and an ability to reduce circulating leptin are stronger predictors of an exercise related response. In terms of the exercise-mediated change in insulin sensitivity, greater responses were observed in volunteers who were most insulin resistant at baseline, who improved leptin sensitivity, and who increased habitual physical activity levels. Only lower body adiposity was associated with an exercise-mediated increase in insulin sensitivity, whilst total body fat oxidation did not influence the change in insulin sensitivity.

Women without a family history of diabetes stimulate improved insulin sensitivity after exercise in concert with reductions in $\text{TNF}\alpha$, leptin, energy intake and increased fasting hepatic fat oxidation however, these changes are likely to be dependent on reduced fat mass and an acute effect of energy deficit. Women with a family history of diabetes increase insulin sensitivity in association with reduced leptin, CRP, lower body fat, resting heart rate and increased physical activity, independent of fat mass. These factors are also independent of the energy deficit of the intervention but not baseline insulin resistance. A putative mechanism which is currently understudied in Offspring subjects is the interaction between exercise and sympathetic hyperactivity. This study is unable to determine the pathways by which these factors might affect baseline insulin resistance, although this information provides more evidence that planned aerobic exercise and increased habitual activity has greater metabolic effects on those with greater metabolic dysfunction related to insulin resistance. Reduced circulating leptin was an independent predictor of improved insulin sensitivity, and was independent of reduced fat mass and baseline insulin sensitivity but not the energy deficit of exercise. Reduced circulating leptin would appear to mediate an insulin-

sensitising effect through a different, but currently incompletely described pathway in Offspring compared to Controls.

Future studies are clearly required to identify the mechanisms by which exercise improves insulin sensitivity, particularly in Offspring where the mechanism may be less dependent on reduced fat mass than in Controls. Detailed imaging to identify changes in adipose tissue compartments and intramuscular fat storage would be of interest, particularly to determine whether the observed association with reduced lower body fat is a marker of intramuscular lipid content. In addition, more detailed assessment of the inflammatory cascade (including TNF α receptor status, CAM assay, adipocyte size and adipose tissue macrophage content and examination of the dynamic function of adipose with respect to NEFA and adipokine efflux) would be of value. This should be paired with measurement of microvascular function to determine the anti-inflammatory effect of exercise. Further exploration of the role of autonomic hyperactivity is a further area of study which theoretically links low grade inflammation and microvascular disease. However, prior to an exercise intervention trial exploration of the cause-and-effect relationship between autonomic activity and inflammation would be required since mechanisms have been suggested which consider both factors as causal (Thayer 2009).

If the primary cause of skeletal muscle insulin resistance is impaired microvascular function (Serne et al. 2006) then improvement in microvascular function in response to exercise would be expected to be closely associated with the magnitude of increased insulin sensitivity (Pedersen 2006). If in addition to this, reduction in both adipokine release from adipose tissue and inflammatory activity in skeletal muscle were observed in concert with improved skeletal muscle capillary recruitment and insulin sensitivity, then this would be indicative of a metabolic process of local and systemic inflammation which is prone to modulation by exercise and which mediates improved insulin sensitivity by increased endothelial function. However whilst this finding would suggest a metabolic pathway which is sensitive to

exercise, it does not describe specific mechanisms which effect the increase in insulin sensitivity.

Description of putative metabolic pathways may allow specific mechanistic studies of the final determinants of change in insulin sensitivity in response to exercise. With respect to the exercise-mediated change in insulin sensitivity in Offspring, the association with reduced circulating leptin represents an important target for further study. Reduction in circulating leptin has consistently been associated with increased insulin sensitivity and increased fat oxidation (Barwell et al. 2008;Corpeleijn et al. 2007;Polak et al. 2006;Solomon et al. 2008). The data in this chapter does not suggest that reduced circulating leptin mediates a direct insulin sensitising effect by increased fat oxidation. Therefore, the mechanism underlying improved 'leptin sensitivity' requires investigation of other 'leptin-centric' mechanisms (such as insulin-stimulated phosphorylation of the AS160 substrate of Akt (Alkhateeb et al. 2009) or AMPK activation (Uotani, Abe & Yamaguchi. 2006;Dyck 2009)) in skeletal muscle after an exercise intervention.

CHAPTER 6

DETERMINANTS OF FAT LOSS IN RESPONSE TO EXERCISE IN PREVIOUSLY SEDENTARY WOMEN

6.1 Introduction

It is well-established that extent of weight or fat loss in response to exercise training varies between individuals (Byrne et al. 2006; King et al. 2008; Snyder et al. 1997). The extent of compliance to the exercise intervention clearly contributes to this variation (Byrne et al. 2006; Colley et al. 2008; McTiernan et al. 2007) but even when this is accounted for, differences in weight and fat loss between individuals are observed (King et al. 2008; Snyder et al. 1997). Failure to achieve predicted weight loss due to alterations in energy expenditure has been termed ‘adaptive thermogenesis’ and its origin is multifactorial, suggestive of both behavioural and metabolic effects (Major et al. 2007).

It has been suggested that individual differences in compensatory adjustments to the increased exercise energy expenditure are responsible for this variability (King et al. 2007). Indeed, it has recently been reported that those who lost less weight than predicted in response to an exercise intervention increased their energy intake over the course of the intervention, whereas those who lost more weight than predicted decreased energy intake, although there was no overall change in energy intake before and after the intervention for the group as a whole (King et al. 2008). Additionally, those who compensate for the energy deficit of exercise by increased energy intake may show greater desire for food after exercise, and may preferentially select energy dense foods after an exercise session (Finlayson et al. 2009). There may also be a difference between compensatory energy intake behaviour in males compared to females in response to a similar exercise programme (McLaughlin et al. 2006).

Furthermore, compensatory decreases in energy expended in spontaneous activity have been shown to lead to smaller than expected increases in total energy expenditure in response to exercise interventions (Goran & Poehlman 1992; Kempen, Saris, & Westerterp 1995) and a wide variation in the extent of change in non-intervention energy expenditure when individuals undergo an exercise-training intervention has been reported (Goran & Poehlman 1992; King et al. 2007). The extent to which this contributes to individual differences in weight or fat loss is unclear, but it has been shown that individuals with the greatest increases in non-exercise activity thermogenesis (NEAT) in response to overfeeding are protected from weight gain (Levine, Eberhardt, & Jensen 1999), which suggests that individual differences in activity compensation could conceivably play a role in determining responsiveness to exercise-induced weight loss.

However, differences in behavioural compensation probably cannot explain all of the variability in responsiveness to exercise-induced weight and fat loss. For example, Bouchard and colleagues reported that in men residing at an isolated experimental station in a highly controlled environment, imposition of an exercise-induced energy deficit of 4.2 MJ per day for 84 days, with constant energy intake, led to reductions in body weight ranging from 3 to 12 kg (Bouchard et al. 1990), a range which is unlikely to be fully explained by differences in compensatory activity between subjects. Relatively homogenous metabolic responses to an exercise intervention were observed in identical twins, compared to other matched pairs, suggesting a constitutive influence on the metabolic response to exercise (Bouchard et al. 1994). Thus non-behavioural metabolic factors are also likely to contribute to individual responsiveness to exercise-induced weight loss. One metabolic factor which could contribute is resting metabolic rate, however, a recent report found no significant difference in the change in resting metabolic rate between the start and end of an exercise intervention between subjects who lost more weight than expected and who lost less weight than expected, suggesting that this does not play a major role (King et al. 2008). A further metabolic factor which might play a role is the magnitude of the exercise-induced change in

resting fat oxidation. Both fasting and postprandial fat oxidation have been shown to increase for at least 24 hours following an exercise session (Burton et al. 2008; Hansen, Shriver, & Schoeller 2005; Votruba et al. 2002), even in the absence of an exercise-induced energy deficit (Burton et al. 2008) and studies have reported greater fat oxidation at rest in endurance-trained versus untrained individuals (Romijn et al. 1993). The exercise-induced increase in resting fat oxidation varies between individuals (Blaak & Saris 2002; Burton et al. 2008; Goodpaster et al. 2003), and indeed the extent of this increase has been demonstrated to be a strong predictor of other exercise-induced metabolic changes, such as the magnitude of change to postprandial lipid metabolism (Burton et al. 2008) and insulin sensitivity (Goodpaster et al. 2003). Furthermore, a number of studies have shown that a high respiratory quotient (RER) (indicating high carbohydrate and low fat oxidation) measured fasting (Marra et al. 1998; Seidell et al. 1992) or over 24 hours (Zurlo et al. 1990) is a significant predictor of long-term weight gain, independent of metabolic rate (Marra et al. 1998; Seidell et al. 1992; Zurlo et al. 1990). Thus effects of exercise on resting fat oxidation may contribute to fat loss in response to an exercise intervention. It is therefore hypothesised that variability in the upregulation of resting fat oxidation following an exercise training intervention would contribute to the inter-individual variability in exercise-induced fat loss and that this effect would be independent of energy expended during exercise training sessions and the effects of behavioural compensatory responses. In addition, this study aimed to determine whether any baseline physiological, metabolic, anthropometric or behavioural characteristics could predict responsiveness to exercise-induced fat loss.

6.2 Methods

6.2.1 Volunteers

Volunteers for this study participated in an intervention trial to determine the effects of exercise training on insulin sensitivity in women with and without a family history of diabetes as described in Chapter 4 (Barwell et al. 2008). They were recruited and screened as described in Section 2.1. Sixty-two women completed the intervention and complete data sets for the variables of interest were available in 55 volunteers. These 55 women were included in the data analysis and their characteristics are presented in Table 6.1. Twenty seven of the women were offspring of patients with type 2 diabetes and 28 had no family history of the disease.

6.2.2 Study design

The overall study design has been described previously in section 2.1.3 but, in brief, subjects all underwent a metabolic assessment, body composition measurements (by Dual X-ray Absorptiometry, DEXA) and a cardio-respiratory fitness test, including individualised calibration of the heart rate versus oxygen uptake relationship, at baseline and after a controlled 7-week endurance-type exercise training programme. Dietary intake and physical activity were monitored during the week preceding baseline metabolic assessment and during the final week of the exercise training programme.

6.2.3 Metabolic assessment

Volunteers reported to the metabolic suite after 12-hour overnight fast and a 20-minute expired air sample was collected using a ventilated hood system (Deltatrac Metabolic Monitor, Datex Engstrom, Kent, UK) to determine resting oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), RER (i.e. $\dot{V}CO_2/\dot{V}O_2$) and metabolic rate (Sections 2.3.2-2.3.3).

Resting heart rate was recorded immediately following the ventilated hood measurement using an automated device (Complior, Artech Medical, Pantin, France) as described in Section 2.3.5. Volunteers completed their final exercise session 15-24 hours prior to the post-intervention measurements.

6.2.4 Body composition assessment

Dual X-Ray Absorptiometry (DEXA) scans (LUNAR Prodigy DEXA scanner, GE Healthcare Diagnostic Imaging, Slough, Berkshire, UK) were used to determine body composition and fat distribution (Section 2.2.5). Height, body mass and waist circumferences were also determined using standard protocols (Sections 2.2.1 – 2.2.4) (Marfell-Jones et al. 2006).

6.2.5 Fitness test and calibration of the heart rate versus $\dot{V}O_2$ relationship

Volunteers attended for this test at least two hours after eating. After a 10-minute rest, 5-minute expired air samples were collected via a mouthpiece into Douglas bags for the determination of $\dot{V}O_2$ and $\dot{V}CO_2$ during three sedentary activities – i.e. sitting, standing, and standing with arms swaying. The mean $\dot{V}O_2$ of these three activities was taken as representative of $\dot{V}O_2$ during sedentary activities (Section 2.5.1). Following this, subjects performed an incremental, submaximal, treadmill walking test to determine their heart rate versus $\dot{V}O_2$ relationship and estimate maximal oxygen uptake ($\dot{V}O_{2max}$) (Section 2.6).

6.2.6 Physical activity and dietary assessment

For seven days preceding each metabolic assessment day, subjects wore a heart rate monitor for all waking hours (Polar 610i; Polar Electro, Kempele, Finland) as described in section 2.9. Average daily heart rate minus resting heart rate was used as a surrogate measure of total

activity and time spent above 1.5 times and 2 times resting heart rate were used as surrogate indices of time spent engaging in activities of at least light and at least moderate intensity, respectively (Section 2.10). During this time subjects also completed 7-day weighed food diaries which were analysed using a computerised version of food composition tables (CompEat Pro; Nutrition Systems, Banbury, UK) (Section 2.8).

6.2.7 Exercise intervention

Volunteers participated in a 7-week endurance type exercise programme, as described in section 2.7. A total of 32 exercise sessions were prescribed over the course of the intervention. Other than participating in the exercise intervention, subjects were requested to make no changes to their lifestyle for the duration of the study.

The energy expended during each exercise session was calculated from the mean exercise heart rate for the session and the individualised heart rate versus $\dot{V}O_2$ relationship as described in section 2.9.2. The net energy expenditure of all exercise sessions was summed to determine the total net energy expenditure of the exercise training programme.

6.2.8 Reproducibility and statistical power

To determine the reproducibility of the resting RER measurements in a free-living situation, 28 of the women in this study underwent two ventilated hood measurements following a 12-hour fast, at an interval of eight weeks, with no specific guidelines other than to maintain their usual diet and physical activity habits throughout the interval between measurements. Fasting RER was 0.84 ± 0.04 on the first visit and 0.85 ± 0.05 on the second visit (NS) and the SD for the difference in RER between visits was 0.05. Based on these data, the present study, with 55 participants, had sufficient statistical power to detect a difference in RER with

exercise training of 0.02 with 90% power. This number of volunteers also enables detection of a correlation between variables of 0.26 at the $p \leq 0.05$ level.

6.2.9 Statistical analysis

Data were analysed using Statistica (version 6.0; StatSoft, Tulsa, OK, USA) and Minitab (version 13.1, Minitab Inc., State College, Pennsylvania). Univariate linear regression analyses were performed to determine the relationship between change in total and regional (trunk, upper body (i.e. trunk plus arm), and leg) fat mass over the course of the intervention and: total net energy expenditure of the exercise intervention; behavioural compensation variables (i.e. change in energy, fat, carbohydrate and protein intake, change in average daily heart rate minus resting heart rate, and change in time spent above 1.5 and 2 times resting heart rate excluding exercise training); changes in physiological variables (i.e. change in $\dot{V}O_{2\max}$, change in resting metabolic rate, change in fasting RER); and baseline physiological and behavioural characteristics (age, BMI, total and regional fat mass, $\dot{V}O_{2\max}$, resting metabolic rate, fasting RER, energy, fat, carbohydrate and protein intake, average heart rate minus resting heart rate, time spent above 1.5 and 2 times resting heart rate). Using the same method as described in Section 3.2.9.2, the extent to which variables were related to change in fat mass independently of exercise energy expenditure was determined. Univariate linear regressions were performed between the residuals for change in fat mass of the regression between change in fat mass and net total energy expenditure of exercise and the other variables. To further adjust for the effect of change in energy intake on change in fat mass, the residual for change in fat mass after regression with net total energy expenditure of exercise and change in energy intake was used in univariate linear regressions with the other variables. Finally, to determine which variables were independent predictors of the change in fat mass, multiple-regression analysis was performed. Statistical significance was accepted at the $p \leq 0.05$ level.

6.3 Results

Baseline characteristics and group changes in response to the exercise intervention are shown in Table 6.1. In response to the exercise training intervention, the group as a whole significantly reduced BMI (by 0.9%), total fat mass (by 3.3%), trunk fat mass (by 3.0%), upper body fat mass (by 3.8%), leg fat mass (by 2.5%), percentage body fat (by 2.6%) and waist circumference (by 1.4%), but lean body mass was not significantly changed. $\dot{V}O_{2\max}$ (by 13.6%) was significantly increased. Resting metabolic rate was not changed but fasting RER was significantly reduced, indicating a shift in substrate utilisation towards fat oxidation. Although volunteers were asked not to alter their diets over the course of the intervention, energy (by 6.3%), carbohydrate (by 7.6%) and protein (by 8.8%) intakes were significantly lower at the end of the intervention than at baseline, but there were no significant differences in fat intake between the start and end of the intervention. Average daily heart rate minus resting heart rate and total time spent above 1.5 times and 2 times resting heart rate (including time spent in the exercise sessions) were all significantly higher at the end of the intervention than at baseline ($p < 0.01$), but the latter two factors did not differ between baseline and the end of the intervention when the time spent during the intervention exercise sessions was excluded.

Table 6.1. Volunteer characteristics at baseline and in response to exercise training.

	Baseline	Change with exercise training
Age (years)	34.7 ± 6.4	-
BMI (kg.m ⁻²)	27.5 ± 4.7	-0.24 ± 0.65**
Lean mass (kg)	41.8 ± 5.8	0.34 ± 1.37
Fat mass (kg)	29.9 ± 9.6	-0.97 ± 1.48**
% body fat	39.3 ± 6.0	-1.02 ± 1.47**
Waist circumference (cm)	85.9 ± 11.9	-1.20 ± 2.26**
VO ₂ max (ml.kg ⁻¹ .min ⁻¹)	31.3 ± 5.1	4.2 ± 4.0**
Insulin sensitivity index	6.79 ± 3.82	0.75 ± 2.33*
Resting metabolic rate (kJ.d ⁻¹)	6250 ± 918	-12 ± 410
Fasting RER	0.853 ± 0.051	-0.030 ± 0.055**
Post-glucose RER	0.948 ± 0.042	-0.035 ± 0.052**
Energy intake (kJ.d ⁻¹)	7904 ± 1787	-500 ± 1530*
Fat intake (g.d ⁻¹)	66.9 ± 20.4	-1.8 ± 19
Carbohydrate intake (g.d ⁻¹)	247.3 ± 72.0	-18.9 ± 53.4**
Protein intake (g.d ⁻¹)	71.6 ± 17.5	-6.3 ± 14.1**
Resting heart rate (beat.min ⁻¹)	68 ± 8	-3.4 ± 5.7
Time above 1.5 times resting heart rate inc.exercise training (min.d ⁻¹)	-	40 ± 11
Time above 2 times resting heart rate inc.exercise training (min.d ⁻¹)	-	23 ± 3
Time above 1.5 times resting heart rate exc.exercise training (min.d ⁻¹)	106 ± 91	9 ± 11
Time above 2 times resting heart rate exc.exercise training (min.d ⁻¹)	11 ± 15	4 ± 2
Number of exercise training sessions completed	-	28.3 ± 6.3
Total duration of exercise training completed (min)	-	1402 ± 469
Mean heart rate during exercise (beat.min ⁻¹)	-	143 ± 9
Total net exercise energy expenditure (MJ)	-	36.9 ± 17.0

N = 55, values are mean ± SD. *p < 0.05 for change with exercise training (**p < 0.01)

Across the group, there was a wide individual variation in change in total fat mass over the intervention, with individual changes in total fat mass ranging from a 2.1 kg gain to a 5.3 kg loss (Figure 6.1).

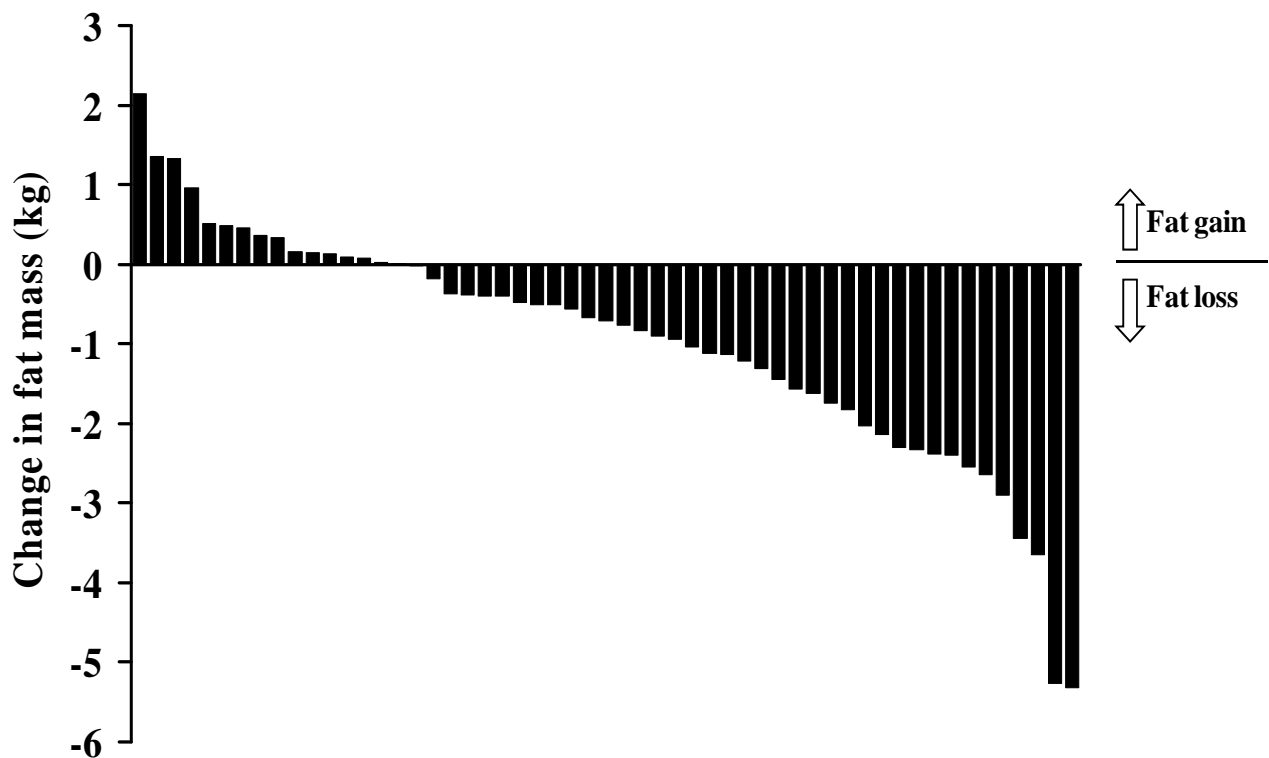


Figure 6.1 Individual changes in fat mass in whole group (n = 55) after exercise intervention. Volunteers ranked in order of change in unadjusted fat mass.

Correlates of the change in fat mass over the intervention are shown in Table 6.2. The strongest correlate of change in total fat mass during simple univariate regression analysis was total net energy expenditure of exercise ($r = 0.60$, $p < 0.0005$), explaining 36% of the variance in this factor. Change in average daily heart rate minus resting heart rate (which incorporates heart rate elevations from the exercise intervention) also significantly correlated with change in total fat mass ($r = -0.31$, $p < 0.05$) but change in time spent over 1.5 and 2 times resting heart rate (excluding exercise training) did not. Change in fat intake ($r = -0.25$, p

= 0.06) and change in fasting RER ($r = -0.25$, $p = 0.05$), had borderline significant correlations with change in total fat mass in simple univariate regression analysis.

Table 6.2. Correlations between interventions, behavioural compensation and physiological variables and change in fat mass in response to exercise training.

		Correlation with change in fat mass		
		No adjustment	After adjustment for total net exercise energy expenditure	After adjustment for total net exercise energy expenditure and change in energy intake
Intervention variables	Total net energy expenditure of exercise	-0.60 (<0.0005)	-	-
Behavioural compensation variables	Change in energy intake	0.20 (0.14)	0.20 (0.14)	-
	Change in fat intake	0.25 (0.06)	0.24 (0.07)	0.10 (0.48)
	Change in carbohydrate intake	0.11 (0.42)	0.17 (0.22)	0.00 (0.99)
	Change in protein intake	0.05 (0.73)	0.05 (0.71)	-0.06 (0.65)
	Change in time spent above 1.5 times resting heart rate	0.06 (0.64)	-0.05 (0.73)	-0.03 (0.85)
	Change in time spent above 2 times resting heart rate	-0.04 (0.75)	-0.10 (0.48)	-0.07 (0.60)
Physiological variables	Change in VO ₂ max	0.03 (0.82)	0.20 (0.14)	0.15 (0.26)
	Change in resting metabolic rate	0.13 (0.33)	0.15 (0.28)	0.15 (0.28)
	Change in Fasting RER	0.25 (0.06)	0.31 (< 0.05)	0.26 (0.05)

N = 55, values are correlation coefficients with p-values in brackets.

Figure 6.2 shows the individual residual changes in fat mass after adjustment for the net total energy expenditure of exercise, and Figure 6.3 shows the individual residual changes in total fat mass after adjustment for the net total energy expenditure of exercise and changes in energy intake. In other words, figures 6.2-6.3 show the variation in change in total fat mass which cannot be explained by changes in exercise energy expenditure and energy intake over the intervention.

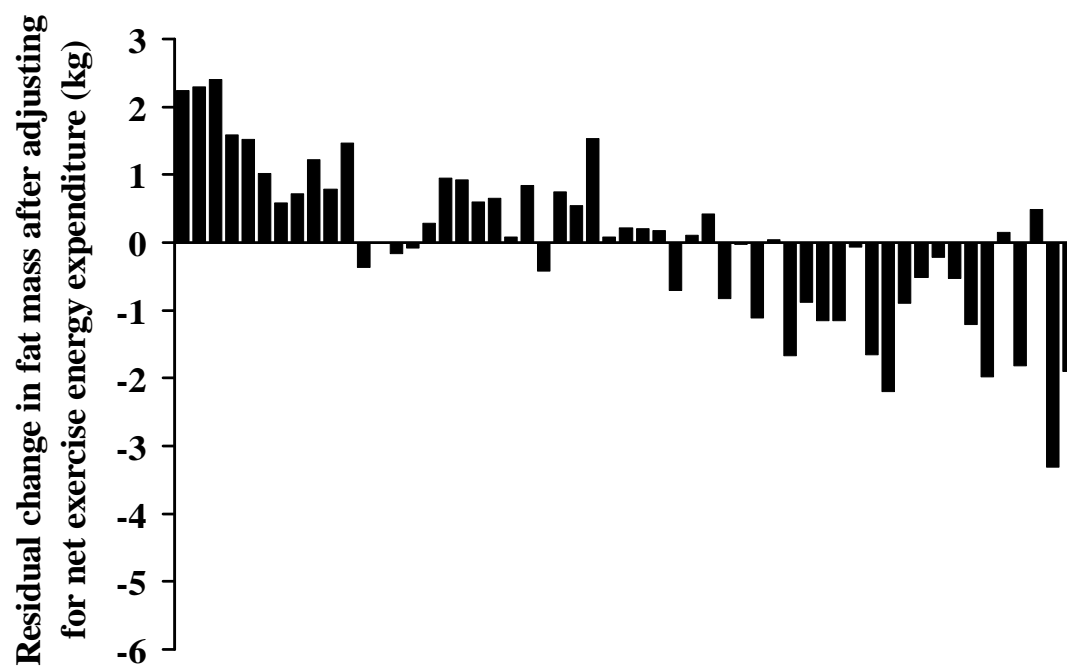


Figure 6.2 Individual residual changes in fat mass for whole group ($n = 55$) after adjustment for net total energy expenditure of exercise intervention. Volunteers ranked in order of change in unadjusted fat mass.

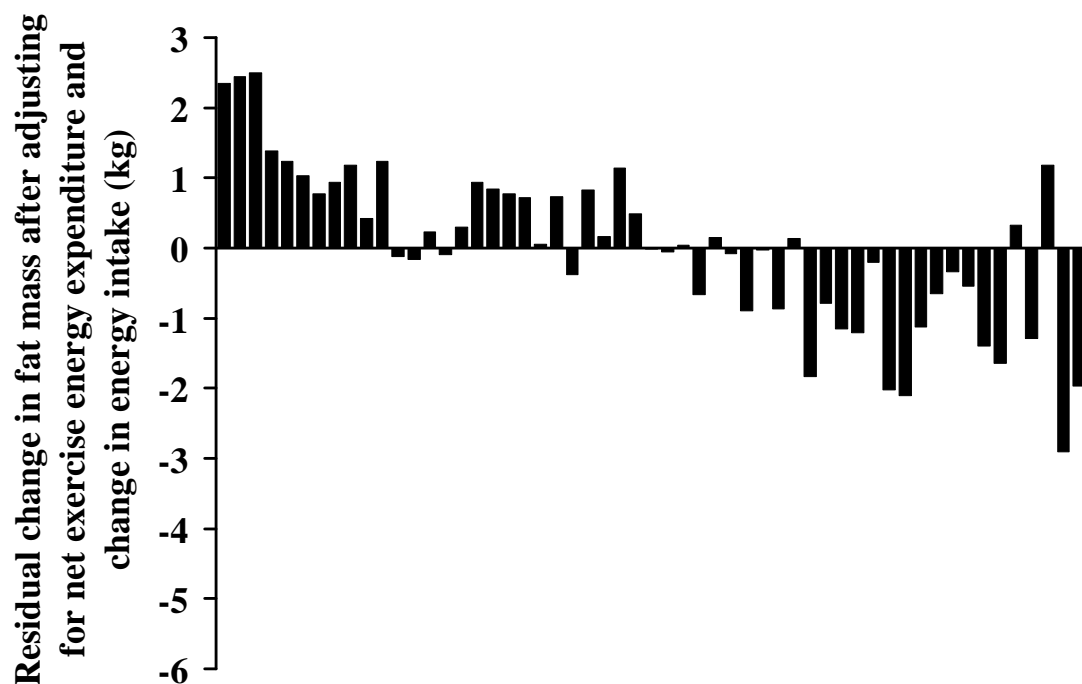


Figure 6.3 Individual residual changes in fat mass for whole group ($n = 55$) after adjustment for net total energy expenditure of exercise intervention and change in energy intake. Volunteers ranked in order of change in unadjusted fat mass.

The relationship between change in total fat mass and change in fasting RER was strengthened after adjustment for the total net energy expenditure of the exercise ($r = -0.31$, $p < 0.05$) and remained after further adjustment for change in energy intake ($r = -0.26$, $p = 0.05$) (Figure 6.4). Thus, change in fasting RER explained ~7% of the variance in the change in total fat mass over the course of the intervention after adjustment for effects of the total net energy expenditure of exercise and change in energy intake. Adjusting for exercise energy expenditure and change in energy intake both weakened the relationship between change in total fat mass and change in fat intake. None of the included baseline factors correlated significantly with change in total fat mass in any of these analyses (data not shown). In multiple-regression analysis including all of the variables described in the statistical analysis section in the model, net total energy expenditure of exercise ($p < 0.0005$) and change in fasting RER ($p = 0.02$) were the only statistically significant independent predictors of

change in fat mass. Together, these two variables explained 40.2% of the variance in change in fat mass (adjusted R^2).

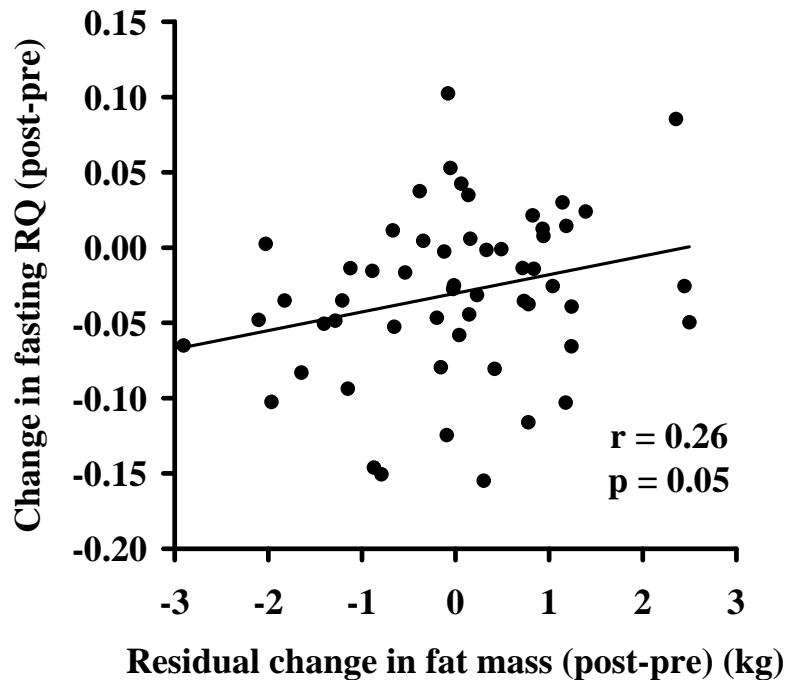


Figure 6.4 Scattergram showing the relationship between change in fasting RER and residual change in fat mass, adjusted for the effects of net energy expenditure of exercise and change in energy intake ($N = 55$, r and p values for univariate linear correlations).

In univariate regression, changes in trunk ($r = 0.87$, $p < 0.001$), upper body ($r = 0.92$, $p < 0.001$) and leg ($r = 0.52$, $p < 0.001$) fat were strongly associated with changes in total fat mass. In addition, changes in trunk ($r = -0.43$, $p < 0.01$), upper body ($r = -0.49$, $p < 0.001$) and leg ($r = -0.45$, $p < 0.01$) fat all significantly correlated with total net energy expenditure of exercise. Change in trunk fat also significantly correlated with change in average daily heart rate minus resting heart rate ($r = -0.29$, $p < 0.05$) and change in upper body fat correlated significantly with fat intake at baseline ($r = -0.28$, $p < 0.05$) (i.e. those with highest baseline fat intake lost the greatest amount of upper body fat). Changes in trunk, upper body or leg fat were not significantly associated with change in fasting RER.

Because the exercise training-induced change in fasting RER was a significant predictor of change in total fat mass, univariate and multivariate regression analyses were performed to determine whether any of the other measured variables could predict the exercise-induced change in RER. Change in fasting RER was significantly associated with diabetes family history (with 0 and 1 included as dummy variables for negative and positive diabetes family history, respectively) $r = -0.35$, $p < 0.01$) (i.e. those with a positive diabetes family history had a bigger exercise-induced reduction in fasting RER); baseline BMI ($r = 0.29$, $p < 0.05$) and total ($r = 0.29$, $p < 0.05$) and leg ($r = 0.36$, $p < 0.01$) fat mass (i.e. those with lower baseline BMI and fat mass had bigger reductions in RER); and changes in energy ($r = 0.29$, $p < 0.05$), carbohydrate ($r = 0.28$, NS) and fat ($r = 0.27$, $p < 0.05$) intake (i.e. those with bigger decreases in energy, carbohydrate and fat intake had bigger reductions in RER). In multiple regression analysis, baseline fasting RER ($p < 0.001$), diabetes family history ($p < 0.001$) and change in energy intake ($p < 0.05$) were independent significant predictors of the change in fasting RER, together explaining 55.9% of the variance. Baseline fasting RER alone explained 44.2% of the variance in the change in fasting RER.

6.4 Discussion

In this study, 55 women underwent a 7-week exercise training programme, which induced a mean total fat loss for the group of 0.97 kg. The mean net total energy expenditure of the exercise programme was 36.9 MJ, so, assuming that fat loss requires a negative energy balance of 39.4 MJ/kg (Elia, Stratton, & Stubbs 2003), fat loss in the group as a whole was broadly at the expected level. However, considering the change in fat mass at group level obscures the wide inter-individual variability in change in fat mass with the intervention (see Figure 6.1). The most important correlate of change in fat mass was net total energy expenditure of exercise, which explained 36% of the variance, but even after adjusting for this and for changes in energy intake over the intervention, a wide variation for the residual change in fat mass was evident, ranging from +2.5 kg to -2.9 kg. The main novel finding of the study was that the residual change in total fat mass in response to an exercise intervention, after adjustment for the energy expended during the exercise intervention and for changes in energy intake was related to the change in fasting RER between the start and end of the intervention. Change in fasting RER explained ~7% of the variance of the residual change in fat mass. Indeed, the relationship between change in fasting RER and change in total fat mass was independent of all other physiological and behavioural variables included in a multivariate analysis model and in this model change in RER and net total energy expenditure of exercise were the only significant independent predictors of change in total fat mass.

It is not possible from the design of this study to determine the direction of causality between change in fasting RER and change in fat mass conclusively. However, the case that exercise-induced reduction in RER was a significant predictor of exercise-induced fat loss fits well with the body of data indicating that a high RER predicts long-term weight gain (Marra et al. 1998; Seidell et al. 1992; Zurlo et al. 1990) – in other words, good fat oxidisers were protected from future weight gain. This data builds on this work, indicating that individuals who had

the largest shifts in resting substrate utilisation towards fat oxidation in response to exercise training experienced the greatest losses in fat mass, independent of exercise energy expenditure and change in energy intake. The studies of RER and long-term weight gain found this effect to be independent of metabolic rate (Marra et al. 1998; Seidell et al. 1992; Zurlo et al. 1990) and the results of this study are consistent with this. Indeed, in agreement with the study of King and co-workers (King et al. 2008), it was found that changes in resting metabolic rate between the start and end of the intervention were not associated with change in fat mass.

On the other hand, it is well established that negative energy balance leads to increased fat oxidation (Pagliassotti et al. 1997) and the women with the greatest fat losses in this study would, by definition, have incurred the greatest negative energy balances over the course of the intervention. However, this shift towards fat oxidation appears to occur largely in response to acute negative energy balance over the relatively short-term, and over the longer term, a number of studies have reported that weight loss (i.e. incurring a large cumulative negative energy balance over a number of weeks or months, with consequent changes in body composition) is often associated with no change (Valtuna et al. 1997) or even an increase (Luscombe et al. 2003; Pasman, Westerterp, & Saris 1999; Weyer et al. 2000) in fasting and 24-hour RER. Indeed, an increase in RER in response to weight loss is thought to be one of the factors predisposing to weight regain following weight loss: a number of reports have indicated that the individuals with the highest RERs following weight loss are those who are most susceptible to weight regain (Froidevaux et al. 1993; Valtuna et al. 1997). Furthermore, the change in fat oxidation in response to the same degree of short-term negative energy balance (Burton et al. 2008), and long-term change in body weight (Weyer et al. 2000), is highly variable between individuals. For example, previously reported individual changes in whole-body postprandial fat oxidation ranged from a decrease of 4 grams to an increase of 16 grams over an 8.5-hour observation on the day following an exercise session inducing an identical energy deficit of 27 kJ.kg⁻¹ in all subjects (Burton et al. 2008). It has been

demonstrated that this variability has metabolic consequences beyond the regulation of body weight: individuals to upregulate post-exercise fat oxidation to the greatest extent in response to a given energy deficit also experience the largest changes to postprandial lipid metabolism (Burton et al. 2008) and insulin sensitivity (Goodpaster et al. 2003). In addition, it has been shown that exercise increases subsequent fat oxidation for at least 24 hours even in the absence of an associated energy deficit and that the extent of this increase also varies markedly between individuals (Burton et al. 2008). As the post-training metabolic assessment was undertaken 15-24 hours after an exercise session, it is likely that the variation in change in RER in the present study at least partly reflects inter-individual differences in the acute post-exercise increase in fat oxidation. Thus, the evidence from the literature indicates that exercise-induced changes in RER exhibit a large degree of inter-individual variability and are evident in the absence of energy deficit, and that RER is not consistently reduced following weight loss, so does not support the rationale that larger reductions in RER are simply the consequence of larger incurred negative energy balances and greater weight losses.

As the change in fasting RER was significantly, independently, associated with change in total fat mass, this study aimed to determine what factors influenced change in RER. By far, the most important predictor of the change in fasting RER was its baseline value – those with the highest baseline values for RER experienced the greatest reductions. Thus, it appears that individuals who are ‘carbohydrate oxidisers’ when sedentary experience the greatest shift towards fat oxidation in response to exercise training. This is an exciting possibility which suggests that those who are most susceptible to weight gain due to their high RER (Marra et al. 1998; Seidell et al. 1992; Zurlo et al. 1990), experience the greatest benefits in terms of increase in resting fat oxidation from exercise. However, baseline fasting RER was not significantly associated with change in total fat mass, a degree of caution is advised when interpreting this observation. Change in fasting RER was also significantly associated with other ‘innate’ baseline factors, namely, BMI, total and leg fat mass and diabetes family

history; however, change in components of dietary intake were also implicated, albeit to a much lesser degree than baseline RER, suggesting that 'behavioural' and 'metabolic' factors influencing change in fat mass are not completely independent of each other. Further investigation in this area could confirm that inter-individual ability to lose weight in response to exercise is predicated on ability to upregulate fasting fat oxidation. Alteration in fasting RER in response to a single exercise session would allow stratification of subjects into potential 'responders' and 'non-responders' prior to an exercise intervention designed to induce weight loss. Such a study would require blinding of the volunteers to their potential exercise response and close control of diet and levels of spontaneous physical activity over the period of the exercise intervention. Actual reduction in fat mass after the exercise intervention would provide evidence that ability to reduce weight in response to exercise is predictable and determined, in part, by innate metabolic response to exercise.

The present study has a number of strengths. With 55 participants, it is the largest study to date attempting to address the issue of factors influencing individual responsiveness to exercise-induced fat loss, which provides statistical high power to detect associations between variables, making the statistical findings of this study robust. In addition, both metabolic and behavioural variables were included in the analyses enabling the relative importance of each to be determined, and energy expenditure of the exercise intervention was objectively quantified on an individual-by-individual basis. Furthermore, baseline and post-intervention testing was performed at an interval of eight weeks to ensure that, as far as possible, women were in the same phase of menstrual cycle for baseline and post-intervention testing and thus limiting the confounding effects of cyclical hormonal changes on the results.

The main limitations to this study, which are common to the majority of reports in this field, relate to the measurement of behavioural compensation variables. Firstly, it is well established that underreporting is a common problem incurred in measurement of dietary

intake, and that this problem is greater in obese than lean individuals (Black & Cole 2001; Westerterp & Goris 2002). However, it appears that the extent of underreporting is relatively consistent within an individual (Black & Cole 2001), implying that differences in dietary intake between two observation points (e.g. baseline and post-intervention) are likely to be determined with greater accuracy than absolute dietary intakes at a single time-point. Thus, the repeated-measures design in the present study may attenuate this potential error. Furthermore, the effect of inaccuracy in measurement of dietary intake would act to diminish any association with exercise-induced change in fat mass (a regression dilution bias effect (Frost C 2000)): thus the finding that there was a borderline significant relationship between change in fat intake and exercise-induced change in fat mass in the present study, despite potential errors in the assessment on dietary intake, suggests that this association was likely to be real. This would be in agreement with the recent work of King and colleagues who reported that dietary compensation influenced the extent of weight loss in response to exercise training (King et al. 2008). In contrast, this study found no evidence to suggest that individual differences in compensation by reducing non-exercise activities influenced the extent of fat loss with the heart rate monitoring methodology employed in the present study. However, because factors other than activity (e.g. excitement and stress) can influence heart rate, particularly when heart rates are relatively low (as they are over most of the day), this approach may have missed subtle changes in activity which could have contributed to responsiveness to exercise-induced fat loss. Thus, it is conceivable that individual differences in compensatory changes in spontaneous physical activity in response to an exercise intervention could have an effect on the extent of exercise-induced changes in fat mass which it was not possible to detect in the present study.

In conclusion, this study found that exercise-induced change in fat loss is associated not only with exercise energy expenditure but also with changes in RER at rest. Thus, development of strategies to maximise the shift in resting substrate utilisation towards fat oxidation in

response to an exercise training programme may help individuals to maximise exercise-induced fat loss.

CHAPTER 7

GENERAL DISCUSSION

7.1 Experimental chapter summaries

The aim of Chapter 3 was to describe the differences between sedentary, premenopausal women with and without a parental history of type 2 diabetes. The findings confirm that familial history of T2D is associated with greater insulin resistance and that this may be mediated by a greater sensitivity to the deleterious metabolic effects of adiposity. Further support for this suggestion may be derived from the finding that although circulating NEFA concentrations were not different between the groups, after adjustment for fat mass Offspring displayed a negative correlation between NEFA and insulin sensitivity. The correlation between adiponectin and insulin sensitivity in Offspring alone may represent an adaptive response towards the effects of NEFA on insulin sensitivity, and the relationship between CRP and insulin sensitivity in Offspring suggests a role for a low-grade inflammation in the development of insulin resistance. Although Offspring subjects were less physically active than Controls, habitual physical activity levels influenced baseline insulin sensitivity in Controls alone. However, it should be appreciated that both the methods used to assess habitual activity and the range of activity observed in the Offspring group may prevent detection of a relationship between physical activity and insulin sensitivity in this group.

Chapter 4 examined the effect of a 7-week aerobic exercise programme on physical and metabolic characteristics of women with, and without a parental history of diabetes. Despite similar compliance to the exercise intervention and similar improvements in cardiorespiratory fitness, women with a parental history of T2D exhibited an augmented metabolic response to exercise, showing increased insulin sensitivity, increased fat oxidation and reduced circulating leptin. Three-day detraining results in Offspring showed increased circulating

leptin and reduced fat oxidation without a change in insulin sensitivity. The persistence of improved insulin sensitivity is consistent with the literature comparing exercise responses in younger and older women (Goulet et al. 2005). Taken together, these results suggest that women with a parental history of diabetes display a more favourable metabolic response to exercise, with relatively improved insulin sensitivity which may be mediated by a metabolic pathway which involves reduction in circulating plasma leptin and increased fat oxidation.

Chapter 5 explored the contribution of changes in body composition, adipose tissue hormones and whole body fat oxidation to improved insulin sensitivity after an exercise intervention. When considered as a single combined group the greatest improvement in insulin sensitivity was seen in those who were most insulin resistant prior to the intervention, those who reduced circulating leptin concentrations, who expended the most energy during the intervention and those who increased habitual physical activity. When considered as a separate group, improved insulin sensitivity in Controls was associated with an increase in hepatic fat oxidation. However although reductions in circulating leptin, TNF α and energy intake were also associated with improved insulin sensitivity, these associations were not independent of reduced fat mass. This may suggest that in Controls, exercise improves insulin sensitivity primarily through reduction in fat mass. Exercise appears to influence insulin sensitivity by different mechanisms in Offspring. These subjects appeared to be more sensitive to the net energy cost of the total intervention which might suggest a more direct dose-response relationship between exercise and insulin sensitivity in this group. Alternatively, it may suggest that Offspring have a lower threshold for exercise-mediated insulin sensitisation. An interesting association between reduced lower body fat and insulin sensitivity was observed in this group, this association was independent of reduced total fat mass. This could suggest that lower body fat is a marker of intramuscular fat, but this would be contrary to the observation that exercise does not necessarily reduce IMCL but does alter partitioning (Dube et al. 2008). Measured by DEXA scan, thigh fat may be a better marker of

deep subcutaneous fat than in other areas of the body (Ryan et al. 2002), particularly the abdomen where it is not possible for the scanner to delineate subcutaneous from visceral fat. If Offspring, like South Asians (Sniderman et al. 2007), are more sensitive to the metabolic influence of deep subcutaneous fat, exercise may preferentially reduce the more metabolically deleterious adipose pools leading to increased insulin sensitivity. Both CRP and resting heart rate were negatively correlated with the post-intervention increase in insulin sensitivity. These factors were independent of reduced fat mass and energy expended during exercise, but not baseline insulin resistance. A link between these two seemingly disparate observations could be made by considering the effects of autonomic hyperactivity. Autonomic hyperactivity is associated with both hyperinsulinaemia and low grade inflammation (Egan 2003) and exercise has been shown to improve insulin sensitivity in concert with improved autonomic responsiveness (Giallauria et al. 2008). However, a similar pattern was also observed with the index of habitual activity (average heart rate minus resting heart rate) and it is possible that a reduction in resting heart rate is also a marker of increased habitual physical activity.

Increased insulin sensitivity in Offspring was associated with reduced circulating leptin and this relationship was independent of alterations in fat mass but not independent of the energy deficit of the intervention. Other authors have suggested that the reduction in circulating leptin mediates improved insulin sensitivity by increased skeletal muscle fat oxidation (Goodpaster et al. 2003; Solomon et al. 2008). Surprisingly, fat oxidation was not associated with the exercise-mediated increase in insulin sensitivity in any of the groups therefore the results of Chapter 5 cannot support this hypothesis. However, if reduced leptin influences insulin sensitivity, but is dependent upon energy deficit then leptin could be acting as a ‘system-wide’ signal for energy deficit and may interact with AMPK to stimulate peripheral insulin sensitivity by mechanisms other than increased skeletal muscle fat oxidation (Kahn et al. 2005; Hutchinson et al. 2008).

The exercise-mediated change in circulating leptin and fat oxidation, combined with the rebound leptinaemia and reduction in fat oxidation in response to detraining, suggests that exercise increases skeletal muscle insulin sensitivity by a mechanism of leptin-sensitive fat oxidation. Increased fat oxidation in skeletal muscle would be expected to reduce lipid intermediates (Dube et al. 2008) and result in improved insulin signalling (Bonen, Dohm, & van Loon 2006). Leptin is recognised to promote fat oxidation in healthy individuals (Minokoshi et al. 2002; Steinberg et al. 2002). If this was the causative mechanism, increased fat oxidation would have been expected to have been significantly associated with the exercise-mediated increase in insulin sensitivity. In Chapter 5 this was not found to be the case. Therefore, increased fat oxidation may be a marker of both improved skeletal muscle insulin and leptin sensitivity, rather than a direct causative factor in the observed increase in insulin sensitivity. Additionally, although exercise may directly influence fat oxidation, the effects on insulin sensitivity may be indirectly mediated through the action of intermediates on insulin signalling (Schenk & Horowitz. 2006; Schenk et al. 2009) which is consistent with the observation that intramyocellular DAG and ceramides are more closely associated with insulin sensitivity than IMCL (Delarue & Magnan. 2007). Leptin-stimulated fat oxidation may reduce intramyocellular intermediates which directly influence peripheral insulin sensitivity. Alternatively, leptin may mediate improved skeletal muscle insulin sensitivity by other mechanisms (Stefanyk & Dyck 2010). Rodent studies have shown that reduced skeletal muscle leptin receptor expression in high-fat fed animals and leptin ‘resistance’ in human myotubes can be overcome by direct AMPK stimulation using AICAR (Dyck 2009). Leptin has also been shown to activate AMPK in hepatic cells (Uotani, Abe, & Yamaguchi 2006). Leptin has been considered to have direct effects on GLUT4 translocation in myotubes *in vitro* (Berti & Gammeltoft 1999) and in palmitate-induced insulin resistance, leptin has recently been shown to stimulate palmitate oxidation and separately promote GLUT4 expression via increased insulin-stimulated Akt activity (Alkhateeb et al. 2009). Therefore if, in addition to the effects of leptin on fat oxidation, leptin activation of AMPK and Akt can

also promote activation of downstream insulin signalling, then the effects of increased leptin sensitivity on insulin sensitivity may be at least two-fold. Adipose tissue expression of the long isoform of the leptin receptor is increased in response to exercise in Offspring compared to Controls and in combination with improved insulin sensitivity and decreased circulating leptin suggests improved leptin sensitivity (Moran et al. 2010). Leptin receptor expression is increased in trained muscle in humans (Olmedillas et al. 2010). If exercise also increases leptin receptor expression in skeletal muscle in Offspring then it could be considered that improved leptin sensitivity results in increased fat oxidation, reduced lipid intermediates and enhanced leptin-mediated stimulation of insulin signalling.

In adipose tissue, leptin is typically considered to be a pro-inflammatory hormone which potentiates release of TNF α and other cytokines which mediate endothelial dysfunction and skeletal muscle insulin resistance (Gil et al. 2007; Lago et al. 2007). In addition, leptin may directly stimulate adipose tissue macrophage infiltration (Gruen et al. 2007; Gutierrez, Puglisi, & Hasty 2009). Therefore if leptin's role in adipose tissue inflammation is solely stimulatory, improved adipose tissue leptin sensitivity might be expected to promote both inflammation and insulin resistance. However, murine studies of adipose tissue inflammation show that exercise is effective in reducing pro-inflammatory cytokine expression in adipose tissue and that this is paralleled by reduced plasma leptin concentrations (Bradley et al. 2008). In addition, Moran and co-workers describe a state of increased 'leptin sensitivity' which is associated with increased insulin sensitivity (Moran et al. 2010). It would seem likely that an improved state of adipose tissue leptin sensitivity is anti-, rather than pro-inflammatory, however the mechanism(s) which underpin this in adipose tissue are not currently known.

Leptin resistance has been described as a failure to increase metabolic rate and energy expenditure and to reduce fat mass despite rising leptin concentrations (Galic, Oakhill, & Steinberg 2010). Many of the models and mechanisms of increased inflammation which are

attributed to hyperleptinaemia are described in obesity and relatively little work has been done in examining the relationship between leptin and adipose tissue inflammation in lean, insulin sensitive subjects. Therefore, similar to initial assumptions about leptin inducing insulin resistance (due to the observed associations between obesity, high leptin and insulin resistance) the suggestion that in all states, leptin promotes inflammation may be incorrect (Stofkova 2009). Since both free and bound leptin appear to be associated with differing metabolic effects (Magni et al. 2005) and leptin is recognised to inhibit AMPK activity centrally, but stimulate it in peripheral tissues (Galic, Oakhill, & Steinberg 2010) it is perhaps not surprising that leptin action within adipose tissue may alter depending on the metabolic state of the tissue. The observation that Offspring have improved exercise-mediated adipose tissue 'leptin sensitivity' would suggest that at least one leptin-related metabolic process is differently exercise-responsive in Offspring compared to Controls. Potential mechanisms include; improved adipose tissue insulin sensitivity leading to lower rates of lipolysis, restoration of the synergistic relationship between leptin and adiponectin on insulin signalling (Stefanyk & Dyck 2010) and a currently unexplained mechanism by which leptin sensitive adipose tissue reduces inflammatory protein production. Further examination of the adipose tissue samples obtained (Appendix B1) would provide further information about adipose tissue inflammation with respect to alterations in adipocyte size and macrophage infiltration in response to exercise. Objective assessment of alteration in adipose tissue inflammatory cell content would contribute to the understanding of a potential differential anti-inflammatory effect of exercise in Offspring compared to Controls. Analysis of components of the insulin signalling pathway, in particular the activity of AMPK and Akt, would also provide further evidence linking exercise, leptin activity and adipose tissue insulin sensitivity.

Chapter 6 examined the determinants of exercise mediated fat loss, particularly with respect to possible mechanisms which compensate for the energy deficit of exercise and lead to a reduction in predicted fat loss. These results suggest that the most important determinant of fat loss in response to exercise remains the energy deficit of the exercise intervention.

Additionally, the novel finding from this study was that the change in fat mass (after adjustment for changes in energy intake and the energy deficit of exercise) was related to the change in fasting RER, suggesting that fat loss is associated with a shift towards fasting fat oxidation. This observation was independent of other variables, suggesting an innate response to exercise which contributes to an individual's ability to reduce fat mass. Those with the highest pre-exercise RER showed greatest reduction in post-exercise RER, providing further evidence that those who are predisposed to weight gain may benefit most from a change from a sedentary lifestyle. However, baseline RER was not significantly associated with fat loss and change in fasting RER was also associated with baseline factors such as diabetes family history. Therefore an 'innate' predisposition to fasting carbohydrate oxidation in a sedentary state and an exercise stimulated shift towards fasting fat oxidation is associated with greater fat loss, independent of the energy deficit of the intervention.

The remainder of this chapter will focus upon areas of interest generated by these studies and potential future avenues of research which may further expand the understanding of the interaction of diabetes family history, exercise and metabolic health.

7.2 Adipose tissue – distribution, function and insulin sensitivity

Results from Chapters 3, 4 and 5 reflect the importance of adiposity in contributing to insulin sensitivity. Increased adiposity is strongly associated with insulin resistance, and the relationship between adiposity and baseline insulin resistance in Offspring suggests an increased sensitivity to adipose tissue in this group. Parallels can be drawn with other groups at high risk of T2D, in particular South Asians (Razak et al. 2007). It is suggested that maximal adipose tissue compartment size may be genetically determined (Chandalia et al. 2007) and that since different adipose tissue pools are recognised to have different metabolic properties (Walker et al. 2007) increased fat mass may have a distinct metabolic impact

which is dependent on the expansion of a specific adipose pool. Central adiposity is recognised to be an important co-founder in the development of T2D however, it is unclear whether visceral fat (Banerji et al. 1999), deep subcutaneous fat or superficial subcutaneous fat (Chandalia et al. 2007; Sniderman et al. 2007) is the most important metabolically active pool, particularly with respect to circulating NEFA and adipose tissue derived hormones. Sniderman and colleagues (2007) suggest that in South Asians a reduction in the size of the subcutaneous adipose compartment leads to relative expansion of deep subcutaneous and visceral pools compared to Caucasian subjects exposed to a similar environment (Sniderman et al. 2007). In Pima Indians, visceral fat mass was not found to be independently associated with insulin resistance (Gautier et al. 1999). Further investigation of the metabolic activity of visceral, deep subcutaneous and superficial subcutaneous fat in Pimas suggests that expanded deep subcutaneous fat is more sensitive to lipolysis than superficial subcutaneous fat and may have greater influence on skeletal muscle insulin sensitivity (Koska et al. 2008b). Additionally, changes in both hyperinsulinaemia and circulating leptin independent of fat mass in response to weight loss suggests that adipose tissue function may be more important than total adiposity (Doucet et al. 2000). It is recognised that basal and stimulated rates of lipolysis differ between superficial and deep adipose tissue pools and that adipokine production (leptin and TNF α) may also be dependent on specific pool volume and local effects of systemic signals (such as catecholamines) (van Harmelen et al. 2002). Detailed comparison of the functional aspects of deep and superficial adipose tissue has not yet been performed (Koska et al. 2008), analysis of rates of lipolysis in basal and stimulated conditions, evidence of local inflammation and adipokine (and receptor) expression in these pools would be of value. In addition, alterations in specific tissue pools in response to exercise would be of interest, particularly when comparing Offspring and Control responses.

Access to visceral fat is limited in a research environment however both visceral and subcutaneous adipose tissue could be obtained in elective surgical patients. Laparoscopic cholecystectomy is a frequently performed, minimally invasive surgical technique which

allows access to the abdominal viscera. This operation is usually an elective procedure and therefore would allow recruitment of subjects to a study. Gallstone disease in the United Kingdom has a high prevalence in young, menstruating women (Shaffer 2006) and it would be possible to recruit age and BMI-matched women planned for elective surgery based on their family history of diabetes and obtain samples of visceral and subcutaneous adipose tissue during the process of their surgery. Whilst this would not add to understanding of exercise-mediated change in adipose tissue, it may provide more information about specific characteristics of adipose tissue morphology and inflammatory protein expression in separate tissue pools and differences between Offspring and Controls.

The supraphysiological dose of glucose required in the OGTT completely suppressed circulating NEFA in both Offspring and Control volunteers. Further investigation is required to understand the influence of adipose tissue on insulin resistance in these groups. In future, *in vivo* subcutaneous fatty acid metabolism in matched Offspring and Control subjects should be studied using the arteriovenous difference technique described by Frayn and colleagues (Frayn, Coppack, & Humphreys 1993). The NEFA and TG response to either a reduced dose (20 grams) of oral glucose or a mixed meal with this technique would provide a more physiological assessment of lipid metabolism in adipose tissue and skeletal muscle (Tan et al. 2008). Increased NEFA efflux from adipose tissue in the post-prandial state promotes hepatic glucose production and hepatic and skeletal muscle steatosis and insulin resistance. Use of these techniques would further describe the role of adipose tissue and NEFA in the aetiology of insulin resistance in Offspring. The use of the hyperinsulinaemic euglycaemic clamp with radiolabelled glucose would be useful to clarify the hepatic and skeletal muscle contributions to total body insulin resistance (DeFronzo, Simonson, & Ferrannini 1982). These techniques are expensive, labour-intensive and more unpleasant for the volunteer than those used in the studies in this thesis but would have the advantage of providing potentially unifying data on the interaction between adipose tissue, liver and skeletal muscle in Offspring and Controls.

Adipokines have autocrine and paracrine activity and this may not be reflected in peripheral concentrations, therefore use of the microdialysis technique with peripheral venous sampling would allow detection of differences in adipokine activity (and possibly the site of activity) between Offspring and Controls (Dostalova et al. 2009;Nielsen et al. 2009). In addition, combining knowledge of adipokine production with direct microscopy of adipose tissue from biopsies would provide a more thorough understanding of the relationship between adipocytes, adipose-bound inflammatory cells and adipokines, particularly if such studies were performed before and after an exercise intervention.

Further exploration of adiposity in sedentary females with a parental history of T2D would be valid. The methods used in the studies contained in this thesis were unable to accurately describe visceral fat or distinguish deep and subcutaneous adipose tissue. Computed tomography (CT), magnetic resonance imaging (MRI) and ultrasonography (US) are able to distinguish between visceral and subcutaneous adipose tissue and by identification of the *fascia superficialis* (Figure 7.1) quantify deep and superficial subcutaneous compartments (Smith et al. 2001). Ultrasound guided placement of deep and superficial subcutaneous microdialysis catheters would allow comparison of adipokine efflux from these two distinct adipose pools (Siklova-Vitkova et al. 2009). This may be of use in determining if the ‘overflow hypothesis’ suggested by Sniderman and colleagues (Sniderman et al. 2007) has metabolic evidence to support the suggestion that deep subcutaneous adipose tissue is more metabolically active than superficial tissue. To date, there does not appear to have been any attempt to utilise ultrasound guidance to sample these compartments.

Kelley and colleagues (2000) observed associations between deep subcutaneous and visceral adipose tissue and both dyslipidaemia and increased insulin and glucose concentrations

which were not apparent for superficial subcutaneous adipose tissue (Kelley et al. 2000). In addition, Smith and co-workers describe sexual dimorphism in distribution of deep and superficial subcutaneous and visceral fat (Smith et al. 2001) and within males and females, ethnic differences in adipose tissue distribution are well recognised (Chandalia et al. 2007; Lear et al. 2007).

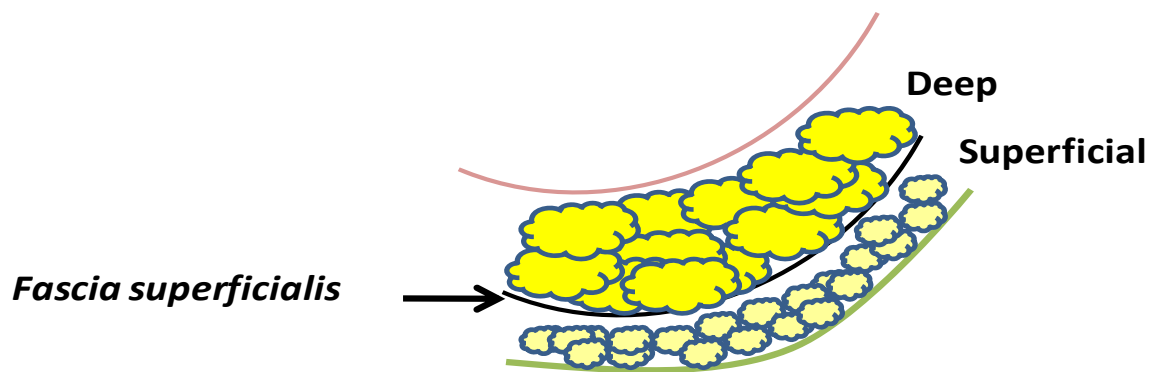


Figure 7.1 Division of deep and superficial subcutaneous adipose by *fascia superficialis*.

Adapted from Smith et al. (2001).

The results described in chapter 5 include an association between improved insulin sensitivity and reduced lower limb adiposity after the exercise intervention. An independent association between lower limb adiposity and insulin resistance has previously been reported (Goodpaster, Thaete, & Kelley 2000; Livingston 2006) and an association between reduced lower limb adiposity and improved insulin sensitivity in response to exercise has been described in only one other study (Goodpaster et al. 2003). Detailed imaging of the lower limb subcutaneous adipose tissue compartments, using modalities described earlier, may be of benefit in investigating these associations. Magnetic resonance imaging of the torso and limbs before and after exercise could determine alterations in subcutaneous and visceral adipose tissue pools. With respect to the lower limb, MRI would give adequate image resolution to assess the degree of subcutaneous and intramuscular adipose deposition. If lower limb fat measured by DEXA is in fact a surrogate for intramuscular adipose content

then more detailed imaging should be able to determine this. Goodpaster and colleagues observed that only the subfascial fat compartment in the thigh was associated with improved insulin resistance, but that CT did not suggest any significant change in intramuscular lipid content (Goodpaster et al. 2003). However the ‘athlete’s paradox’ would highlight that IMCL content is less important than lipid intermediate burden in determining insulin sensitivity (Dube et al. 2008). Therefore direct assessment of the lipid content of lower limb muscle would be required to determine whether exercise is associated with a more ‘pro-metabolic’ profile of intramuscular lipid. Vastus lateralis muscle biopsy allows access to skeletal muscle and has the benefit of being the specific tissue of interest in this case. In addition to determining the overall lipid content of the muscle, thigh biopsy would also allow analysis of lipid droplets and their physical relationship to mitochondria which is thought to be closely associated with peripheral insulin sensitivity (Shaw et al. 2009). Thigh muscle biopsy before and after an exercise intervention in matched Offspring and Controls would provide information on fibre type, lipid and intermediate content and distribution and could also be analysed for the activity of components of the insulin signalling cascade (Cochran et al. 2010). If it were possible to combine these studies with subcutaneous adipose biopsies then the analysis of adipokine receptor expression in both tissues would be of great value in describing the relationship between adipose tissue and skeletal muscle. The author notes the challenge of maintaining adherence to a study which proposes adipose and muscle biopsy, assessment of adipose tissue function by arteriovenous assessment or microdialysis (\pm ultrasound guided sampling of deep tissue), use of the euglycaemic clamp and a seven week exercise intervention. In addition the additional cost of more complex imaging and the technical burden of the studies would require both an extravagant grant and a very sympathetic ethics committee.

The importance of adipose tissue function in determining insulin sensitivity and exercise mediated changes in insulin sensitivity was discussed in Chapter 3, 4 and 5. In each study adipose tissue activity influenced insulin sensitivity independently of fat mass. At baseline,

circulating NEFA concentrations were negatively associated with insulin sensitivity, independent of fat mass and adiponectin was correlated with insulin sensitivity in Offspring alone. In Chapters 4 and 5, the contribution of fat oxidation and reduced circulating leptin to the exercise mediated increase in insulin sensitivity in Offspring supports the hypothesis that exercise affects insulin sensitivity through altered adipose tissue function. The multivariate analysis in Chapter 5 did not find fat oxidation to be a significant factor in the exercise-mediated improvement in insulin sensitivity. This observation is surprising in view of the significant role of reduced leptin in improved insulin sensitivity. Arteriovenous differences in substrate utilisation can be measured across skeletal muscle (Kelley et al. 1999) and this may be a more specific method of examining the relationship between insulin sensitivity, leptin and fat oxidation. The association between adiponectin and insulin sensitivity was only observed at baseline. Adiponectin is now recognised to have more than one active form, and at least two distinct receptors (Yamauchi & Kadowaki 2008) therefore the assay used in these studies may not have been specific enough to detect alterations in specific isoforms of adiponectin. Interestingly, Polak and colleagues (2007) observed increases in adiponectin multimers after dietary induced weight loss, but these were not associated with the observed change in insulin sensitivity (Polak et al. 2007). Abdominal subcutaneous adipose samples were obtained by liposuction biopsy before and after the exercise intervention. Some analysis of these samples has been performed and the increase in adipose tissue 'leptin sensitivity' has been discussed in Section 7.1 (Moran et al. 2010). As previously described, microscopy of the adipose tissue samples with respect to adipocyte size and inflammatory cell infiltration would provide important information about adipose tissue inflammation and the effects of exercise.

Minimally invasive imaging techniques such as magnetic resonance spectroscopy (MRS) offer real-time description of metabolic function in both muscle (Carey et al. 2003) and liver (Ravikumar et al. 2008). Functional skeletal muscle imaging using techniques such as proton MRS offer the opportunity to examine changes in IMCL in response to exercise (Machann,

Stefan, & Schick 2008), whilst ^{31}P MRS is a marker of skeletal muscle mitochondrial function (Meex et al. 2010). These methods allow minimally invasive assessment of a specific aspect of skeletal muscle function but alone, cannot provide enough information to determine the mechanism of improved insulin sensitivity in response to exercise. Instead, MRS is frequently utilised in concert with such methods as muscle biopsy and indirect calorimetry in an attempt to define the metabolic pathway of interest (Meex et al. 2010).

7.3 Lifestyle factors and implications for weight loss and insulin sensitivity

Habitual physical activity was considered to be important in all experimental chapters. At baseline, contrary to the findings of Higgins and co-workers (2005), Controls displayed a relationship between physical activity and insulin sensitivity which was not observed in Offspring despite a lower level of habitual activity. Increased habitual physical activity was also positively associated with change in insulin sensitivity after exercise in analysis of the combined Offspring and Control group. It is appreciated that the method of assessment of habitual physical activity utilised in these studies is crude. Unfortunately, attempts to assess energy expenditure using the FLEX method were unsuccessful and prevented detailed examination of energy expenditure. Higher levels of habitual physical activity are thought to be protective against chronic diseases such as T2D (Helmrich et al. 1991) and cardiovascular disease (Franco et al. 2005), and may be able to compensate for inherited or acquired tendencies to these diseases (Laaksonen et al. 2003; Gill & Malkova 2006; Gill & Cooper 2008).

In Chapter 6 increased fat loss was observed in subjects who had increased habitual physical activity. Therefore, more robust assessment of physical activity is warranted both at baseline and during an exercise intervention to determine the dose-response relationship between the degree of inactivity and deleterious metabolic perturbations and to examine the exercise

associated alteration in lifestyle which may contribute to, or mitigate against both reversal of these metabolic defects and weight loss.

Despite the problems encountered with the FLEX method on this occasion, it remains a well validated (Livingstone et al. 1990) and relatively simple technique. Prior to further use of the FLEX process, further investigation is warranted into the methodology utilised in the pilot study described in Chapter 2. Comparison with the doubly-labelled water technique would be technically complicated and expensive, but would allow definitive assessment of the validity of the technique used in Chapter 2 and validate any adjustments to the methodology.

In all studies participants were asked to maintain their usual dietary habits. However, at baseline (Chapter 3) and after the exercise intervention (Chapter 4), no differences were observed between Offspring and Controls with respect to either energy intake or dietary composition. In those who participated in the analysis of fat loss (Chapter 6), a significant reduction in energy intake was observed ($500 \pm 1530 \text{ kJ.d}^{-1}$, $p < 0.05$) after exercise. It is recognised that self-reported dietary intake is prone to misreporting, but that these errors are reasonably consistent within individuals (Black & Cole 2001). It is therefore likely that individuals who under- or over-report dietary data will have done so before and during, the exercise intervention. It would be difficult to achieve more accurate data on energy intake in free living individuals over a seven week period. It is however important for future exercise studies to continue to examine energy balance to ensure that results are not confounded by non-study alterations in energy balance. Understanding of the role of macronutrient intake in the development of insulin resistance in people at risk of diabetes is likely to require not only accurate recording of dietary intake, but also greater understanding of the impact of innate and environmental influences on subsequent fat and carbohydrate metabolism (Bergouignan et al. 2009).

In Chapter 6 the ability to reduce fasting RER in response to exercise was a significant determinant of fat loss over the course of the intervention. The propensity to switch from fasting carbohydrate oxidation to fat oxidation in response to exercise, independent of the energy deficit of a seven week intervention suggests a metabolic mechanism which is exercise-sensitive. However, the study described in Chapter 6 raises further questions. Firstly: What is the exercise-sensitive process which is stimulated in these individuals? Secondly: Can the baseline fasting RER response to a single session in sedentary women predict fat loss response to an exercise intervention and furthermore, can this information allow practical individualised advice on exercise prescription for weight loss? Studies to answer the first question would require more detailed assessment of the *in vivo* oxidative function of skeletal muscle. Application of the arteriovenous difference method of substrate utilisation (Kelley et al. 1999) in a skeletal muscle group could provide specific data on the metabolic response in muscle, rather than in whole body. Magnetic resonance spectroscopy using ^{31}P would be a less invasive method of examining the muscle oxidative capacity in subjects who reduce fasting RER. Additionally, since substrate utilisation is dependent on substrate delivery to tissues, an assessment of skeletal muscle microvascular function is warranted. After review of the literature, the author's preference would be to employ contrast enhanced ultrasound, however if less invasive and expensive techniques were more appropriate venous occlusion plethysmography would be an alternative (Clark 2008).

Finally, the relationship between reduced resting heart rate and increased insulin sensitivity after the exercise intervention warrants further exploration. Resting heart rate may simply be a crude indicator of increased fitness or increased habitual activity and therefore a more robust assessment of habitual activity could be helpful in determining if this is the case. The alternative explanation, that resting heart rate is a surrogate for autonomic dysfunction, could be explored by examining heart rate recovery after exercise and it is likely that the current fitness testing protocol could be adapted to allow this to be included (Yeckel et al. 2009).

7.4 Results in women – implications for future studies

7.4.1 General considerations

The findings described in this thesis are exclusively applicable to women, furthermore the majority of the participants in the studies were European Caucasians. These factors have important implications for both future research and any potential public health message. With respect to both of these points it would clearly be beneficial to perform similar studies with sedentary male participants. The mechanisms and mediators of the development of insulin resistance and atherosclerosis, as well as the outcomes from the diseases themselves would appear to differ between the sexes (Engberding & Wenger 2008; Evangelista & McLaughlin 2009; Kilhovd et al. 2005). Observational evidence also suggests that exercise may induce different metabolic changes in women compared to men (Perreault et al. 2004; Soto et al. 2008). In addition, exercise responses in women may be dependent upon the menstrual phase (Northoff et al. 2008) which supports the methods used in this study of test-retest intervals which would be expected to align with menstrual cycle in the majority of women or an alternative method which utilises hormone analysis to dictate testing.

Larger numbers of participants and a longer period of exercise intervention may be desirable for future studies however from the author's perspective the practical considerations of maintaining volunteer involvement and compliance in such a labour intensive process would pose further problems. The remaining sections of this thesis will therefore consider specific studies which would seek to advance understanding of the mechanisms of firstly; increased insulin resistance in Offspring subjects, secondly; the metabolic processes which underpin the observed 'hyper-response' in those who are most insulin resistant and finally; the reasons why a switch from fasting carbohydrate oxidation to fat oxidation in response to exercise might promote fat loss. The process of performing these studies, the data analysis and

preparation of the thesis has led the author to conclude that there is no such thing as a ‘perfect study’ and therefore the suggestions in the three final sections of this thesis are made with an appreciation of financial cost, the practicality of proposed methods and the burden on both the investigator and most importantly, the volunteers.

7.4.2 Insulin resistance in Offspring

The hypothesis that insulin resistance in Offspring is in part determined by adipose tissue derived factors would appear to be correct. However, to consider adipose tissue in isolation is clearly inadequate and with respect to the aetiology of insulin resistance the interaction with the liver, skeletal muscle and vascular endothelium are of particular importance in exploring the mechanisms which confer insulin resistance in Offspring. In retrospect, PWV may have been an inadequate surrogate for endothelial function, particularly in insulin-sensitive subjects. Future baseline assessment of matched Offspring should consider alternative techniques such as contrast enhanced ultrasound to provide minimally invasive assessment of the skeletal muscle microcirculation. It is likely that microvascular dysfunction is an early abnormality in insulin resistance and that it may be a key cofounder in the process (Rattigan et al. 2006). It would be reasonable to consider using a lower dose of oral glucose to prevent the degree of hyperinsulinaemia and NEFA suppression that was found in Chapter 3, accompanied by repetition of the Deltatrac measurement at 1-hour post glucose in an attempt to detect subtle differences in diet-induced thermogenesis between Offspring and Controls. Whilst the ‘gold standard’ euglycaemic clamp would potentially provide distinct data on muscle and liver insulin sensitivity, it is questionable whether the expense, complexity and tolerability of the procedure is worth the additional data in the context of the other investigations proposed. Since skeletal muscle is the major determinant of total body insulin sensitivity more extensive investigation of skeletal muscle is warranted. Whilst non invasive techniques such as CT/MRI and MRS can provide detailed anatomical and limited functional data, muscle biopsy would be the preferred investigation. This would allow comparison of

muscle fibre type, IMCL content and droplet location, measurement of lipid intermediates, adipokine (particularly leptin) receptor expression and perhaps most importantly analysis of the activity of the components of the insulin signalling cascade. The continued use of abdominal adipose biopsies would allow a unification of the role of the adipokine locally and in skeletal muscle. Direct examination of inflammatory cell infiltration and cytokine (and receptor) expression in adipose tissue and muscle, with evidence of cytokine-mediated insulin signalling dysregulation (e.g. Akt inactivation) in muscle and impaired capillary recruitment would be strongly supportive of a link between adipose tissue-derived low grade inflammation and skeletal muscle insulin resistance.

7.4.3 Augmented metabolic response to exercise

The primary hypothesis of Chapters 4 and 5 was that Offspring would show an augmented metabolic response to exercise. This was considered to have been correct. Improved insulin sensitivity was accompanied by increased fat oxidation and a reduction in circulating leptin. Adipose tissue studies before and after an exercise intervention suggest that Offspring display increased ‘leptin sensitivity’ compared to matched Controls (Moran et al. 2010). Again, consideration of adipose tissue in isolation prevents exploration of a mechanism which is likely to have resulted in increased skeletal muscle insulin sensitivity. Muscle and adipose tissue biopsy before and after the exercise intervention would be of value. Evidence of improved muscle leptin sensitivity allied to increased activity of components of the insulin signalling cascade which are considered to be leptin responsive would be a strong indication of a mechanism by which leptin mediates increased insulin sensitivity without stimulating fat oxidation. In practice it would be very difficult to separate the effects of exercise on fat oxidation and insulin signalling from any effects of leptin, particularly as leptin may also upregulate skeletal muscle fat oxidation and reduction in ceramides might also stimulate activation of downstream insulin signalling. Ideally, the administration of a short-acting potent malonyl CoA mimic prior to the muscle biopsy would be expected to ‘switch off’ fat

oxidation and clarify the role of increased leptin expression on insulin signalling. However, the author is not aware of any such compound and the likely toxicity of such a substance would probably preclude its use in human subjects.

For the same rationale as at baseline, post-exercise assessment of adipose tissue inflammation and skeletal muscle capillary function would be of value. Description of adipose tissue compartment response to exercise would be achieved using MRI, this would minimise radiation exposure to a group of relatively young women, whilst providing accurate data on the visceral, superficial subcutaneous and deep subcutaneous adipose pools. With respect to Sniderman and colleagues' (2007) 'overflow' hypothesis, exercise would be expected to preferentially reduce visceral or deep subcutaneous tissue, whilst leaving superficial tissue intact (Sniderman et al. 2007). If MRI were too expensive or unavailable, a single operator could be trained to use ultrasound to assess deep and superficial subcutaneous pools. In addition, identification of the fascia superficialis may allow selective sampling of adipokines by microdialysis although it is unlikely that many volunteers would agree to this in addition to muscle and adipose biopsies.

Assessment of the impact of an exercise intervention in free-living individuals is potentially confounded by alterations of diet and habitual activity. The limitations of dietary reporting have been discussed in Section 7.3. The assessment of habitual activity was limited by the problems with the FLEX method (Chapter 2). Prior to another intervention, review of the protocol for assessment of resting heart rate measurement at baseline should be considered in an attempt to ensure that the initial FLEX heart rate is a true representation of the inflexion point between sedentary and active energy expenditures. In addition, it would be appropriate to incorporate assessment of heart rate variability and/or heart rate recovery as markers of autonomic function (Carnethon et al. 2003). Associations between autonomic dysfunction and low-grade inflammation have been described (Seematter et al. 2004), but not in Offspring

subjects. A causal relationship is unlikely to be determined by these simple measurements but as in patients with polycystic ovarian syndrome (Giallauria et al. 2008), evidence of an exercise interaction with these factors would be worthy of further study.

7.4.4 Determinants of exercise-mediated fat loss

In Section 7.3 it was suggested that there were two potential studies which should expand on the work described in Chapter 6. Firstly, an attempt should be made to explore the association between exercise-mediated reduction in RER and fat loss. Robust assessment of total and regional adiposity should be performed and specific assessment of skeletal muscle, as well as total body, substrate oxidation would determine whether the change in fasting RER is evidence of metabolic flexibility (Kelley et al. 1999). Assessment of carbohydrate oxidation using ^{13}C or fatty acid oxidation using ^{31}P could be performed using MRS (Machann, Stefan, & Schick 2008). Alternatively, cannulation of leg vessels could also be combined with cannulation of the superficial epigastric vein to examine NEFA efflux and muscle substrate utilisation (Frayn et al. 1993; Kelley et al. 1999). The impact of exercise on endothelial function is of interest, since the ability to alter substrate oxidation requires adequate delivery of substrate to the tissue. Therefore contrast enhanced ultrasound would allow assessment of skeletal muscle capillary recruitment (Coggins et al. 2001). If there is evidence of microvascular dysfunction further investigation of inflammatory indices and NO bioavailability would be required since inflammatory cytokines such as $\text{TNF}\alpha$ reduce NO activity (Rattigan et al. 2006). Reduced NO action has endothelial effects but also interferes with insulin signalling, this may be a mechanism which promotes over-reliance on carbohydrate metabolism but the effects of exercise can bypass this insulin signalling interference and IL-6 release by skeletal muscle may be able to reverse the effects of $\text{TNF}\alpha$ (Pedersen 2006).

Secondly, a more clinical application could be a prospective study to determine whether reduction in fasting RER in response to a single exercise session can predict fat loss over a subsequent exercise intervention. In this study, both the principle investigator and the volunteers would need to be blinded to their predetermined status as either ‘responders’ or ‘non-responders’, to prevent bias affecting the subsequent exercise intervention. If the primary hypothesis of this study was found to be correct, it would have important implications for the treatment of overweight and obesity. The cardiovascular benefits of increased physical activity have been recognised since the ground breaking epidemiological studies by Paffenbarger and Morris (Morris et al. 1980;Paffenbarger, Jr. et al. 1986). However, from a weight-loss perspective it may be of benefit to ‘non-responders’ to avoid becoming demoralised by lack of weight loss in response to moderate exercise and conversely, it may stimulate greater compliance with exercise advice if ‘responders’ are aware that they are metabolically predisposed to exercise-mediated weight loss. In the current economic climate health care resources are under increasing pressure to provide ‘value for money’. Targetting exercise advice and intervention towards people with proven insulin resistance, women with a first-degree relative with T2D or overweight people who show metabolic response to exercise may, in all three situations, represent an effective use of resource.

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APPENDICES

Appendix A: Information forms for patients and general practitioners, consent forms and screening questionnaires.

Appendix B: Methods used to obtain subcutaneous abdominal adipose sample by liposuction biopsy technique. Exercise planner. Food diary. Physical activity diary

Appendix C: 'Delayed Exercise' results. Absolute change in variables after seven-week period of 'usual lifestyle'.

APPENDIX A1

HEALTH SCREEN FOR STUDY VOLUNTEERS

(version 3, 11/08/2004)

Name:

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

1. At present, do you have any health problem for which you are:

- | | | |
|--|-----------|----------|
| (a) on medication, prescribed or otherwise | yes [] | no [] |
| (b) attending your general practitioner | yes [] | no [] |
| (c) on a hospital waiting list | yes [] | no [] |

2. In the past two years, have you had any illness which required you to:

- | | | |
|---|-----------|----------|
| (a) consult your GP | yes [] | no [] |
| (b) attend a hospital outpatient department | yes [] | no [] |
| (c) be admitted to hospital | yes [] | no [] |

3. Have you ever had any of the following:

- | | | |
|--------------------------|-----------|----------|
| (a) Convulsions/epilepsy | yes [] | no [] |
| (b) Asthma | yes [] | no [] |
| (c) Eczema | yes [] | no [] |
| (d) Diabetes | yes [] | no [] |
| (e) A blood disorder | yes [] | no [] |
| (f) Head injury | yes [] | no [] |
| (g) Digestive problems | yes [] | no [] |
| (h) Hearing problems | yes [] | no [] |

7. Do you currently smoke yes [] no []

[illegible]

If so, for how long did you smoke and when did you stop?

8. How many units of alcohol do you typically drink in a week?

If YES to any question, please describe briefly if you wish (e.g. to confirm whether problem was short-lived, insignificant or well controlled.) (Use a separate sheet if necessary)

Name and address of GP

Blood pressure measured at screening.....mm Hg

APPENDIX A2

Version 3, 8th June 2004

VOLUNTEER INFORMATION SHEET

Effects of a physical activity programme on insulin resistance and the function of adipose (fat) tissue in women with and without a family history of type 2 diabetes.

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?

The children of parents with type 2 (late-onset) diabetes are themselves at increased risk of developing diabetes and, although not diabetic, often have changes in their metabolism making insulin less effective at controlling their blood sugar levels. This can lead to an increased risk of heart disease and type 2 diabetes. This study will investigate whether a programme of moderate exercise can be effective in normalising the function of insulin in these individuals and will help us to understand how exercise can reduce someone's risk of developing type 2 diabetes and heart disease. The entire study will take 3 years but each person will be involved in the project for about 10 weeks.

Why have I been chosen?

You have been chosen because you are a healthy women aged between 20-45 years who either has at least one parent with type 2 diabetes or has no family history of type 2 diabetes.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

This is a randomised trial to determine how effective exercise will be at improving the function of insulin in the body and reducing risk for late-onset diabetes and heart disease. You will be asked to either undertake a 7-week programme of moderate exercise (exercise group), or to maintain your normal lifestyle (i.e. undertake no planned exercise) for 7-weeks, before undertaking the same 7-week moderate exercise programme as the exercise group (delayed exercise group). Results from the exercise and normal lifestyle periods will then be compared. You will be assigned to the exercise or delayed exercise group by chance – i.e. you cannot choose which group you go into.

What do I have to do?

1) In the first instance you will be asked to attend for a screening visit in which we will:

- discuss with you and complete confidential questionnaires regarding your health, family history, physical activity and diet
- measure your blood pressure
- provide an opportunity for you to ask questions
- take a small blood sample (20 ml or 4 teaspoons) to check the fat and sugar levels in your blood. We will also check for any abnormalities in the function of your liver, kidneys and thyroid gland to ensure that it is perfectly safe for you to participate in this study.

After this visit you will be assigned to the exercise or the sedentary group.

2) We will then ask you to undertake a number of tests to determine your level of health and fitness. These will include:

- **Exercise tests** – The first test will involve walking on a treadmill with self-adhesive electrodes attached to your chest to monitor how hard your heart is working. This test will last a maximum of 12 minutes and will confirm whether it is safe for you to take part in this study. We will then ask you to perform a second test where you will walk or run on a treadmill (depending on your fitness level) at different speeds and we will monitor your heart rate and collect the air you breathe out to determine your fitness level. We will also take tiny ‘finger prick’ blood samples during this test, which will not involve a maximal effort and will last about 20 to 30 minutes.
- **Monitoring physical activity and diet** – we will ask you to weigh and record everything that you eat and drink for a week and to record all your activities during this period in a diary. We will also ask you to wear a heart rate monitor during all your waking hours this week (except for in the shower/bath) and use this information to calculate how many calories you burn during normal daily living. To enable us to do this calculation, we will need to measure your heart rate and collect the air you breathe out during a range of activities (such as lying, sitting, standing and walking) in the laboratory. You should allow about 2 hours to complete these laboratory tests.
- **Assessment of body composition** – we will measure your weight and height and measure around your waist and your hips. We will also perform a scan of your body to

calculate how much fat you are carrying. These tests will take about 15 minutes in total and can be carried out on the same day as other tests.

- **Glucose tolerance test** – we will ask you to come to the laboratory after an overnight fast and drink a sugary drink. We will take blood samples before the drink and for two hours after the drink to assess how your body deals with this sugar. This will help us to determine how well the insulin in your body is working. This will be no more painful than a simple blood test as samples will be obtained from a tiny plastic tube called a ‘cannula’ placed in a forearm vein. We will take approximately 120 ml (about an eggcup full) of blood over the course of this test.
- **Fat biopsy** – when you come to the laboratory for your glucose tolerance test, we will also take a small fat sample from your tummy to study how exercise influences the production of various factors influencing the metabolism of fat tissue. This may help us to understand the way in which exercise can reduce risk of diabetes and heart disease. The fat biopsy will be taken by ‘liposuction’ and involves numbing the skin with a local anaesthetic then sucking up 1 to 2 grams of fat (about the size of a pen top) with a needle and syringe. This is no more painful than having a blood sample taken.

3) If you have been assigned to the exercise group, you will then begin your 7-week exercise programme. Your exercise programme will be individually designed according to your current level of fitness, starting with a small amount of exercise in the first week and building up to a maximum of 5 sessions per week by the end of the programme. The exercise sessions will all be at an intensity which is comfortable for you. You will perform your exercise sessions in the newly re-furnished Glasgow University sports facilities at the Stevenson Building and the Garscube Sports Complex. These are open from 7:30am until 10pm from Monday to Friday, from 9am until 6pm on Saturdays and from 10am until 6pm on Sundays, so you can schedule your exercise sessions to fit in with your other time commitments. You will meet with an investigator at least once per week for a supervised exercise session and to discuss your exercise goals for the following week. It is important that you maintain your usual eating habits during your exercise programme. At the end of the programme you will undergo the same set of tests that you did at the start of the study, i.e. exercise tests, monitoring physical activity and diet, assessment of body composition, glucose tolerance test and fat biopsy. The glucose tolerance test and fat biopsy will be performed twice at the end of the programme; once on the day following your final exercise session and again three days later. This will help us to determine how long the benefits of exercise last when you stop exercising.

4) If you have been assigned to the delayed exercise group, we will ask you to maintain your usual dietary and exercise habits (i.e. undertake no planned exercise) for this 7-week period. At the end of this period you will undergo the same set of tests that you did at the start of the study, i.e. exercise tests, monitoring physical activity and diet, assessment of body composition, glucose tolerance test and fat biopsy. You will then be asked to undertake the same 7-week exercise programme as the exercise group and ONE further set of tests will be performed at the end of this period.

What are the possible disadvantages and risks of taking part?

- Exercise testing will not be at a maximal level but the possibility exists that, very occasionally, certain changes may occur during or shortly after the tests. They include abnormal blood pressure, fainting or a change in the normal rhythm of the heartbeat.

- Blood sampling via the cannula may cause minor bruising, an inflammation of the vein or haematoma (a small accumulation of blood under the skin). Good practice, however, minimises this risk. Some people may feel faint when they give blood.
- Taking the fat biopsy carries a small risk of minor bruising or an infection. Good sterile practice reduces this risk.
- The scan to determine your level of body fat uses a very small dose of radiation. Each scan is equivalent to about 1/20 of a chest X-ray or the amount of natural background radiation that we are exposed to in 4 hours. However, if you are pregnant, or think you might be pregnant, you should inform us as this radiation could harm an unborn baby.
- There is a small possibility that taking part in this study will reveal a health problem that you already have such as high cholesterol or high blood pressure. If such a problem is revealed, we will inform your GP to ensure that you receive appropriate treatment. If necessary, a consultant physician (Dr Steve Cleland) will supervise any follow-up care.

What are the possible benefits of taking part?

Diabetes is a major and growing health problem in Scotland and understanding how we can prevent diabetes is important to individuals at risk of diabetes, the health service and the wider economy. This study aims to increase understanding about how exercise influences the function of insulin, which is an important factor in the development of diabetes. The findings will be published in scientific journals so that understanding of the way in which exercise decreases the risk of diabetes can be increased. This information may help make up better exercise guidelines, particularly for those with parents who have type 2 diabetes.

We will provide you with feedback about the main study findings and also about your own results and would be delighted to explain results and discuss the implications with you.

What if something goes wrong?

The chance of something going wrong are extremely small. All of the procedures involved in this study are low risk and our screening tests are designed to ensure that you will only participate if it is safe for you to do so. In the unlikely event that you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms may be available to you.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the University will have your name and address removed so that you cannot be recognised from it.

Who is organising and funding the research?

This study is being funded by the British Heart Foundation.

What will happen to my samples after the study has finished?

The blood and fat tissue samples that you provide for this study may be useful for future research into the prevention and treatment of diabetes and heart disease; this may involve analysis of certain genes associated with these diseases. Any use of your samples for future research will require further approval from a Research Ethics Committee and samples will be analysed in such a way that the results will not be directly traceable to you. If you do not wish your samples to be used for future research, please indicate this on the consent form.

Who has reviewed the study?

This study has been reviewed and approved by the North Glasgow NHS Trust Research Ethics Committee.

Contact for Further Information

Any questions about the procedures used in this study are encouraged. If you have any doubts or questions, please ask for further explanations by contacting Dr Nicholas Barwell on 07966 434471 /0141 330 6588 or emailing Nick.Barwell@tiscali.co.uk or N.Barwell@bio.gla.ac.uk.

You will be given a copy of this information sheet and a signed consent form to keep for your records.

APPENDIX A3



**UNIVERSITY
of
GLASGOW**

Volunteer Identification Number for this trial:

CONSENT FORM

Title of Project: Effects of a physical activity programme on insulin resistance and the function of adipose (fat) tissue in women with and without a family history of type 2 diabetes.

Name of Researcher: _____

Please initial box

1. I confirm that I have read and understand the information sheet dated 8 June 2004 (version 3) for the above study and

☐

have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical

☐

care or legal rights being affected.

3. I agree to take part in the above study.

☐

4. I agree for my samples to be used for future research into the prevention and treatment of diabetes and heart disease. This may involve analysis of genes associated with these diseases.

Yes

☐

No

☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

APPENDIX A4

Name

Address 1

Address 2

Date

RE:

Dear Dr

Your patient, Mrs, has kindly volunteered for a British Heart Foundation funded clinical trial based at the Institute of Biomedical and Life Sciences in the University of Glasgow. The title of this trial is 'Physical activity, insulin resistance and adipose tissue gene expression in the offspring of patients with type 2 diabetes'. We aim to assess whether a programme of moderate exercise will produce greater changes in insulin sensitivity and adipocyte gene expression in people with a family history of diabetes compared with matched control subjects. The findings may help inform future public health strategies for diabetes prevention.

I enclose a copy of the subject information sheet, and your copy of the patient's consent form. I also enclose details of the initial screening results for your records. I hope to be in contact with you as the study progresses, because some of the detailed physiological, biochemical and anthropometrical measurements that we make over the course of the study may be of some use to you.

If, in the course of screening and testing, we uncover factors which warrant medical input or follow up, these will be discussed with Dr Steve Cleland (Consultant Physician, Diabetes and Endocrinology – Stobhill Hospital) and we will notify you. If you have any questions or wish to contact me for further information, I would be delighted to assist by telephone, email, letter or in person.

Yours sincerely

Dr Nick Barwell MRCP

APPENDIX B1

Adipose tissue was required to provide a sample for adipose tissue gene expression. Sampling was performed at between the hours of 09:00 and 10:00, (if the subject's metabolic test had commenced at 08:00) or between the hours of 11:00 and 12:00 (if the subject's metabolic test had commenced at 10:00). The subject was still fasting at the time of the biopsy, and had been reclining in the supine position for approximately 1 hour prior to the biopsy.

Before the biopsy was performed, subjects were asked about a previous history of allergic reaction to local anaesthetic, iodine, adhesive dressings and metals. The biopsy was performed by a qualified medical practitioner, the sample was processed by an assistant under the supervision of the medical practitioner. Having ensured no contraindication to the procedure, the subject's abdomen was exposed, draped with a sterile towel and the skin cleaned with Betadine solution (Seton Healthcare Group, Oldham, UK). An area of approximately 5 cm by 5 cm was anaesthetised with 10mls of 1% plain lignocaine (Braun Melsungen AG, Melsungen, Germany). The anaesthetised area was approximately 5 – 10 cm lateral to the umbilicus. After a 5 minute period to allow the anaesthetic to take effect, the adipose sample was taken from the anaesthetised area.

The sample was obtained using a 14G Braun Sterican needle (Braun Melsungen AG, Melsungen, Germany) by liposuction technique. This technique relies on the creation of a vacuum by the application of negative pressure to a 20ml syringe attached to the biopsy needle. After insertion of the needle subcutaneously, negative pressure was applied to the syringe, creating a constant suction pressure. The subcutaneous adipose tissue was released by a gentle rocking motion with the needle to break up the aggregates of adipose. The adipose sample was drawn up the needle into the syringe by the negative pressure.

Due to the narrow gauge of the biopsy needle, only a small amount of adipose could be obtained with one pass of the needle. Less than 2mls of sample tissue could be obtained from one pass. The sample was ejected from the needle on to a prepared tub covered with muslin. The assistant washed the sample with physiological saline (0.9% Saline for Irrigation, Baxter Healthcare, Glasgow, UK) and separated the adipose tissue from other tissues with forceps.

In order to increase the adipose yield, the same puncture site was used on three further occasions to attempt to obtain 1g of adipose tissue. Once washed and separated, the adipose tissue was divided into 3 portions and placed in labelled eppendorfs, then immediately snap frozen in liquid nitrogen. In all cases the optimum time from removal of the tissue from the body to snap freezing was less than 5 minutes.

After completion of the biopsy, a sterile dressing was applied to the puncture site, and pressure over the area applied with an ice pack. Pressure was maintained for 10 minutes to encourage the internal aspect of the area to seal and minimise bruising and bleeding. After snap freezing, the adipose samples were stored in a -80°C freezer.

APPENDIX B2

EXERCISE PLANNER

Week No. 6

Week Beginning 6.2.06

Subject Name

TARGET HEART RATE : 140 - 160

Aims

Achieve Target Heart Rate for 60 minutes, 5 times in the next week.

Day	Time of Day	Exercise Performed
Monday		
Tuesday		
Wednesday		
Thursday		
Friday		
Saturday		
Sunday		

Remember!

Wear your heart rate monitor and watch for each exercise session.

Try to keep your heart rate in the target zone for the whole period of your exercise.

Remember to press the big red button twice to begin your exercise session.

Your next meeting with an exercise consultant is

ON : _____

AT : _____ **WITH :** _____

Any problems – phone Nick on 07966 434471

APPENDIX B3

FOOD INVENTORY INSTRUCTIONS

It is important that you weigh and record everything that you eat and drink for each day of your monitoring week.

Please (i) start a separate page for each day.

(ii) start a separate line for each item.

Column 1

Record meal and time and place of eating.

Column 2

Describe each item as accurately as possible, stating where relevant:

(i) type and brand

(ii) whether food is fresh, dried, canned, frozen, salted, smoked, etc.

(iii) whether food is cooked, if so give method of cooking e.g. fried, baked, etc.

Column 3

Record the weight of each item after cooking:

(i) place scales on a level surface

(ii) place plate or container on top of scales

(iii) press 'ON/Reset' button to turn on scales

(iv) once zero appears, add first item of food

(v) record weight displayed

(vi) press reset button before weighing next item

Wherever possible, record weights in grams. If this is not possible, record weights in household measures (e.g. sugar or jam in teaspoons, stating whether level, rounded, or heaped).

Column 4

Record the weight of any leftovers, such as food remaining on plate, weight of container in which food has been weighed, apple cores, etc.

Columns 5 and 6

Please leave blank.

If food consists of several items, please list each on a separate line i.e. instead of writing 'one cheese sandwich', record separately the weights of bread margarine, cheese, etc.

Please remember to record all drinks, as well as food, giving weights where possible, or volumes if these are known. Record separately the weights of added milk and sugar.

An example is shown overleaf.

Food Inventory - Example

Name _____ Date _____

1. Time/Place	2. Description of food/drink	3. Weight of food/drink (g)	4. Weight of container/ leftovers (g)	Leave Blank	
Breakfast	Cornflakes (Kelloggs)	28			
8:30am	Milk (Sainsbury's virtually fat-free)	48			
Home	Bread (Mothers Pride, large white	76			
	sliced, toasted)				
	Flora margarine	7			
	Robinsons lemon marmalade	12			
	Coffee (instant)	2			
	Milk (whole pasteurised)	10			
Lunch	Cheese (Cheddar)	55			
1:00pm	Bread (white, crusty)	76			
Pub	Butter	4			
	Chutney (2 teaspoons)				
Snack	Coffee (instant)	2			
3:30pm	Coffee-mate	6			
Office	Mars Bar	35			
	Apple	76	8 (core)		
Dinner	Turkey Fillet (frozen, grilled)	102			
6:30pm	Potatoes, old, boiled	320	74		
Home			(leftover)		
	Peas (Birds Eye, frozen, boiled)	50			
	Heinz tomato ketchup	14			
	Yoghurt (Ski strawberry thick and	162	10		
	creamy)		(carton)		
	Coffee, filter	148			
	Milk (Sainsbury's virtually fat-free)	8			
Snack	Banana	107			
7:45pm	Orange Tango (can)	330			
Home					

APPENDIX B4

ACTIVITY DIARY

The diary is split up into individual days. Each day is split into 15minute intervals. The boxes are large enough that you should be able to fill in a few things if required. From my point of view, the more detailed you can manage the better, but I would be happy if you can fill as much as possible. A spare day is provided.

You may not want to carry these diaries about with you if you are out. In this case feel free to write things down on smaller areas of paper and transfer them at the end of the day. As most people are sleeping at nights, the time periods are 30mins apart.

The first page is a sample morning. You can see there are lots of ways to let me know what you are doing, and cut corners if you're doing the same thing for a prolonged period.

Any questions – give me a call on 07966433471.

Thanks

Dr Nick Barwell

Sample Day Date 23/9/04

<u>Time</u>	<u>ACTIVITY</u>
0600	Sleep
0615	Sleep
0630	Sleep
0645	Sleep
0700	} Got up, Showered, brushed teeth etc
0715	
0730	Dressed and made bed
0745	Breakfast in kitchen
0800	Tidy up flat
0815	Check bags and diary (sitting down)

0830	Watch news
0845	“ “
0900	Walk to work
0915	Meeting with volunteer
0930	Tests
0945	Tests in lab (Standing and walking)
1000	“ “ “
1015	“ “ “
1030	“ “ “
1045	Work at desk
1100	↓
1115	
1130	Work at desk
1145	“ “ “
1200	“ “ “
1215	Toilet and drink break – walk to toilet
1230	Lunch at desk

APPENDIX C

Table C.1 Absolute changes in physical characteristics and body composition of Control and Offspring after period of ‘usual lifestyle’. Values are Mean \pm SEM.

	Controls	Offspring	P - value
	(n=16)	(n=13)	
Body mass (kg) ^a	0.18 \pm 0.25	0.52 \pm 0.30	0.56
BMI (kg.m ⁻²) ^a	0.066 \pm 0.091	0.191 \pm 0.111	0.56
Total fat mass (kg)	-0.08 \pm 0.29	-0.28 \pm 0.38	0.67
Body fat (%)	0.12 \pm 0.32	-0.82 \pm 0.26	< 0.05
Trunk fat mass (kg) ^a	0.10 \pm 0.17	-0.24 \pm 0.39	0.24
Leg fat mass (kg) ^a	-0.26 \pm 0.13	-0.12 \pm 0.15	0.61
Arm fat mass (kg) ^a	0.13 \pm 0.11	0.09 \pm 0.27	0.42
Android fat mass (kg)	0.02 \pm 0.05	0.01 \pm 0.08	0.92
Gynoid fat mass (kg)	-0.12 \pm 0.07	0.03 \pm 0.08	0.24
Total lean mass (kg)	-0.32 \pm 0.26	0.94 \pm 0.31*	< 0.01
Trunk lean mass (kg)	-0.13 \pm 0.23	0.79 \pm 0.19*	< 0.01
Leg lean mass (kg)	-0.08 \pm 0.12	0.10 \pm 0.14	0.34
Arm lean mass (kg) ^a	0.03 \pm 0.05	0.05 \pm 0.13	0.90
Upper Body Fat (kg) ^a	0.23 \pm 0.18	-0.14 \pm 0.37	0.15
Lower Body Fat (kg) ^a	-0.26 \pm 0.13	-0.12 \pm 0.15	0.61
Waist Circ (cm) ^a	0.36 \pm 0.46	-0.98 \pm 0.43	< 0.05
Hip Circ (cm) ^a	-0.64 \pm 0.41	-0.36 \pm 0.85	0.83
Biceps Skinfold (mm) ^a	0.99 \pm 0.50	-1.90 \pm 1.98	< 0.05
Triceps Skinfold (mm) ^a	-0.42 \pm 0.46	1.70 \pm 1.59	0.12
Subscapular Skinfold (mm)	0.02 \pm 0.75	2.38 \pm 1.80	0.46
Suprailiac Skinfold (mm) ^a	0.59 \pm 0.79	-1.62 \pm 2.41	0.30

Column P-values are for the interaction term in a 2-way ANOVA (group x trial) with repeated measures on the ‘trial’ factor. ^astatistical analysis performed on logarithmically transformed data. *p < 0.05, **p < 0.01, ***p < 0.001 for difference from baseline within group.

Table C.2 Absolute changes in glucose, insulin, NEFA and 3-OHB in the fasted state and during OGTT and insulin sensitivity index after period of ‘usual lifestyle’. Values are Mean \pm SEM.

	Controls	Offspring	P - value
	(n=16)	(n=13)	
Glucose			
Fasting (mmol.l ⁻¹)	-0.04 \pm 0.07	-0.03 \pm 0.08	0.93
Mean during OGTT (mmol.l ⁻¹)	-0.77 \pm 0.42	0.08 \pm 0.19	0.16
Change from fasting during OGTT (mmol.l ⁻¹)	-0.48 \pm 0.27	0.10 \pm 0.21	0.18
Insulin			
Fasting ^a (mU.l ⁻¹)	0.20 \pm 0.38	0.42 \pm 0.71	0.44
Mean during OGTT ^a (mU.l ⁻¹)	-0.58 \pm 4.01	-12.86 \pm 5.22	0.20
Change from fasting during OGTT ^a (mU.l ⁻¹)	-0.78 \pm 3.75	-13.28 \pm 4.83	0.17
NEFA			
Fasting (mmol.l ⁻¹)	-0.08 \pm 0.04	0.02 \pm 0.04	0.05
Mean during OGTT ^a (mmol.l ⁻¹)	-0.03 \pm 0.03	-0.002 \pm 0.02	0.36
Change from fasting during OGTT (mmol.l ⁻¹)	0.07 \pm 0.02	-0.02 \pm 0.02	0.20
3-OHB			
Fasting (mmol.l ⁻¹) ^a	-0.02 \pm 0.02	-0.03 \pm 0.03	0.24
Mean during OGTT (mmol.l ⁻¹)	-0.003 \pm 0.005	-0.01 \pm 0.02	0.60
Change from fasting during OGTT (mmol.l ⁻¹)	0.02 \pm 0.01	0.02 \pm 0.02	0.99
Insulin sensitivity index^a	0.71 \pm 0.78	0.00 \pm 0.49	0.25

Column P-values are for the interaction term in a 2-way ANOVA (group x trial) with repeated measures on the ‘trial’ factor. ^astatistical analysis performed on logarithmically transformed data. *p < 0.05, **p < 0.01, ***p < 0.001 for difference from baseline within group.

Table C.3 Absolute change in fasting adipokines after period of ‘usual lifestyle’. Values are Mean \pm SEM.

	Controls	Offspring	P - value
	(n=16)	(n=13)	
Adiponectin (ng.ml ⁻¹) ^a	0.08 \pm 0.33	0.75 \pm 0.42	0.55
Leptin (pg/ml ⁻¹) ^a	1.01 \pm 1.71	1.53 \pm 1.32	0.43
TNF- α (pg.ml ⁻¹) ^a	0.02 \pm 0.04	0.05 \pm 0.07	0.33
IL-6 (pg.ml ⁻¹) ^a	0.58 \pm 0.28	0.02 \pm 0.15	0.14
Resistin (ng.ml ⁻¹)	-0.25 \pm 0.73	0.70 \pm 0.64	0.35
CRP (mmol.l ⁻¹) ^a	-0.03 \pm 0.31	0.83 \pm 0.37	0.58

Column P-values are for the interaction term in a 2-way ANOVA (group x trial) with repeated measures on the ‘trial’ factor. ^astatistical analysis performed on logarithmically transformed data. *p < 0.05, **p < 0.01, ***p < 0.001 for difference from baseline within group.

Table C.4 Absolute change in fasting liver enzymes and lipids after period of ‘usual lifestyle’. Values are Mean \pm SEM.

	Controls	Offspring	P - value
	(n=16)	(n=13)	
Fasting ALT (mmol.l ⁻¹) ^a	-1.11 \pm 1.37	0.67 \pm 1.46	0.52
Fasting GGT (mmol.l ⁻¹) ^a	0.06 \pm 1.05	6.27 \pm 2.31*	0.06
Total cholesterol (mmol.l ⁻¹)	-0.07 \pm 0.13	-0.03 \pm 0.10	0.82
Fasting triglycerides (mmol.l ⁻¹) ^a	-0.08 \pm 0.04	0.10 \pm 0.06	< 0.05
HDL cholesterol (mmol.l ⁻¹)	0.00 \pm 0.04	-0.01 \pm 0.04	0.88
LDL cholesterol (mmol.l ⁻¹)	-0.05 \pm 0.10	-0.06 \pm 0.09	0.86

Column P-values are for the interaction term in a 2-way ANOVA (group x trial) with repeated measures on the ‘trial’ factor. ^astatistical analysis performed on logarithmically transformed data. *p < 0.05, **p < 0.01, ***p < 0.001 for difference from baseline within group.

Table C.5 Absolute change in metabolic rate, respiratory exchange ratio (RER), rates of fat and carbohydrate (CHO) oxidation in Controls and Offspring in the fasted state and 2 hours after glucose load, after period of ‘usual lifestyle’. Values are Mean \pm SEM.

	Controls	Offspring	P - value
	(n=16)	(n=13)	
RMR (kJ.kg ⁻¹ .day ⁻¹)	0.18 \pm 0.29	-0.07 \pm 0.34	0.58
Fasting RER	0.02 \pm 0.01	0.01 \pm 0.01	0.75
Fasting fat oxidation rate (g.kg ⁻¹ .day ⁻¹)	-0.10 \pm 0.11	0.08 \pm 0.14	0.32
Fasting CHO oxidation rate (g.kg ⁻¹ .day ⁻¹)	0.22 \pm 0.25	0.19 \pm 0.30	0.92
Post-glucose metabolic rate (kJ.kg ⁻¹ .day ⁻¹)	0.13 \pm 0.24	0.05 \pm 0.33	0.85
Post-glucose RER	0.01 \pm 0.01	0.02 \pm 0.01	0.40
Post-glucose fat oxidation rate (g.kg ⁻¹ .day ⁻¹)	-0.03 \pm 0.12	-0.17 \pm 0.11	0.41
Post-glucose CHO oxidation rate (g.kg ⁻¹ .day ⁻¹)	0.03 \pm 0.29	0.73 \pm 0.35	0.13

Column P-values are for the interaction term in a 2-way ANOVA (group x trial) with repeated measures on the ‘trial’ factor. ^astatistical analysis performed on logarithmically transformed data. *p < 0.05, **p < 0.01, ***p < 0.001 for difference from baseline within group.

Table C.6 Absolute changes in fasting systolic and diastolic blood pressure and peripheral PWV in fasted, and 2-hour post-glucose states, after period of ‘usual lifestyle’. Values are Mean \pm SEM.

	Controls	Offspring	P - value
	(n=16)	(n=13)	
Systolic blood pressure (mm Hg)	1.19 \pm 2.66	-7.00 \pm 6.21	0.21
Diastolic blood pressure (mm Hg)	-6.38 \pm 2.27	-5.85 \pm 3.55	0.90
Fasting PWV (m.s ⁻¹) ^a	-0.26 \pm 0.21	-0.21 \pm 0.37	0.87
Post-glucose PWV (m.s ⁻¹) ^a	-0.72 \pm 0.40	0.34 \pm 0.90	0.69

Column P-values are for the interaction term in a 2-way ANOVA (group x trial) with repeated measures on the ‘trial’ factor. ^astatistical analysis performed on logarithmically transformed data. *p < 0.05, **p < 0.01, ***p < 0.001 for difference from baseline within group.

Table C.7 Absolute change in cardiorespiratory fitness and indices of habitual physical activity after period of ‘usual lifestyle’. Values are Mean \pm SEM.

	Controls	Offspring	P - value
	(n=16)	(n=13)	
VO ₂ max ^a (ml.kg ⁻¹ .min ⁻¹)	-0.11 \pm 0.86	-1.58 \pm 0.59	0.19
VO ₂ at LT (ml.kg ⁻¹ .min ⁻¹) ^a	-1.17 \pm 0.65	-0.49 \pm 1.12	0.62
Resting HR ^b (b.min ⁻¹)	0.82 \pm 1.15	1.80 \pm 1.35	0.59
Average daily HR (b.min ⁻¹)	-0.31 \pm 1.20	-1.07 \pm 1.04	0.65
Average daily HR /resting HR	-0.03 \pm 0.03	-0.06 \pm 0.03	0.48
Time spent above 1.5 times RHR (min.day ⁻¹) ^a	17.90 \pm 28.02	-50.41 \pm 16.56	0.20
Time spent above 2 times resting HR (min.day ⁻¹)	3.73 \pm 6.17	-7.99 \pm 3.59	0.15

Column P-values are for the interaction term in a 2-way ANOVA (group x trial) with repeated measures on the ‘trial’ factor. ^astatistical analysis performed on logarithmically transformed data. *p < 0.05, **p < 0.01, ***p < 0.001 for difference from baseline within group.

Table C.8 Absolute change in energy and macronutrient intake after period of ‘usual lifestyle’. Values are Mean \pm SEM.

	Controls	Offspring	P - value
	(n=16)	(n=13)	
Energy (kJ.kg ⁻¹ .day ⁻¹)	-4.26 \pm 2.80	2.83 \pm 3.32	0.10
Total fat (g. kg ⁻¹ .day ⁻¹)	-0.13 \pm 0.10	0.10 \pm 0.10	0.73
SFA (g. kg ⁻¹ .day ⁻¹) ^a	0.01 \pm 0.12	0.03 \pm 0.05	0.85
MUFA (g. kg ⁻¹ .day ⁻¹)	-0.04 \pm 0.03	0.03 \pm 0.04	0.81
PUFA (g. kg ⁻¹ .day ⁻¹) ^a	0.00 \pm 0.02	0.02 \pm 0.01	0.47
Total CHO (g. kg ⁻¹ .day ⁻¹) ^a	0.11 \pm 0.38	-0.55 \pm 0.41	0.45
Sugar (g. kg ⁻¹ .day ⁻¹) ^a	-0.13 \pm 0.23	-0.44 \pm 0.17	0.25
Starch (g. kg ⁻¹ .day ⁻¹) ^a	-0.07 \pm 0.28	0.24 \pm 0.17	0.82
Protein (g. kg ⁻¹ .day ⁻¹) ^a	-0.17 \pm 0.10	0.00 \pm 0.05	0.56

Column P-values are for the interaction term in a 2-way ANOVA (group x trial) with repeated measures on the ‘trial’ factor. ^astatistical analysis performed on logarithmically transformed data. *p < 0.05, **p < 0.01, ***p < 0.001 for difference from baseline within group.